FIG. 1

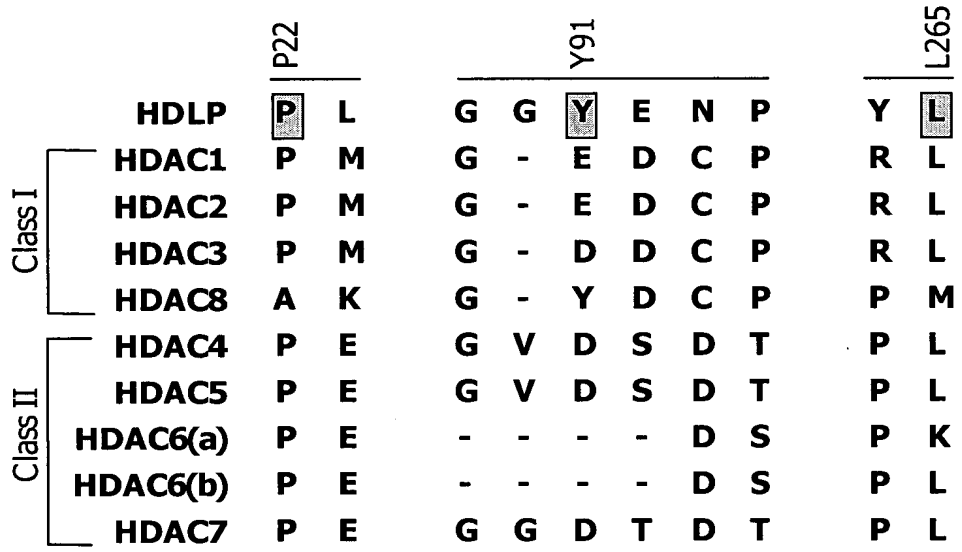

P22
Y91
L265
HDLP P L G G Y E N P Y L
Class I
HDAC1 P M G - E D C P R L
HDAC2 P M G - E D C P R L
HDAC3 P M G - D D C P R L
HDAC8 A K G - Y D C P P M
Class II
HDAC4 P E G V D S D T P L
HDAC5 P E G V D S D T P L
HDAC6(a) P E - - - - D S P K
HDAC6(b) P E - - - - D S P L
HDAC7 P E G G D T D T P L

FIG. 2

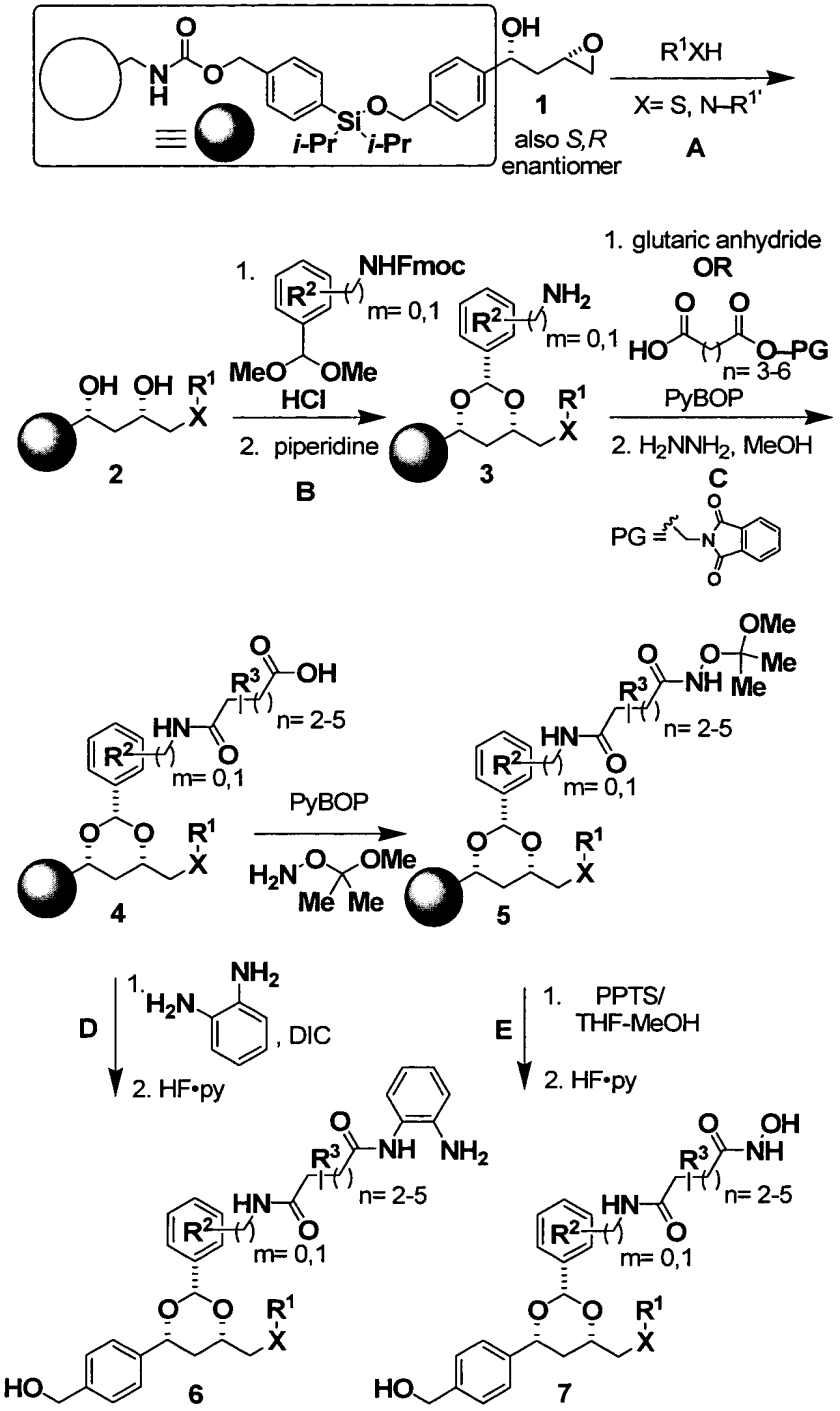
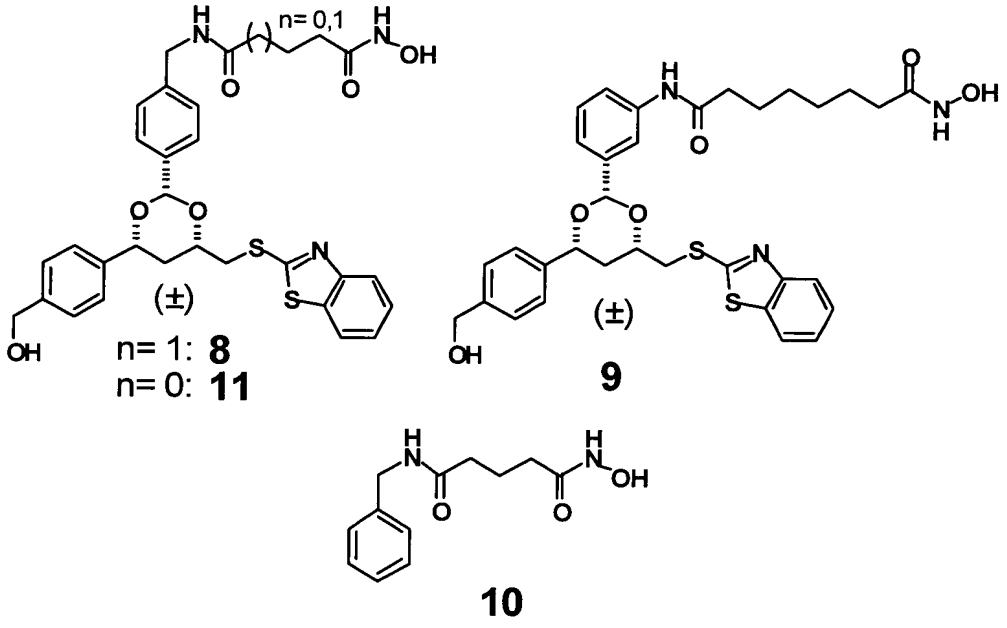

1
also S,R enantiomer
R1XH
X= S, N–R1'
A
1. glutaric anhydride
1. NHFmoc
m= 0,1
NH2
m= 0,1
OR
HO
O–PG
n= 3-6
MeO OMe
HCl
2. piperidine
B
PyBOP
2. H2NNH2, MeOH
C
PG
2
3
n= 2-5
m= 0,1
PyBOP
OMe
Me
4
5
D
1.
, DIC
2. HF•py
E
1. PPTS/ THF-MeOH
2. HF•py
6
7
HO

FIG. 3



Compound	HDAC1	HDAC6
8	1.2 ± 0.5	0.9 ± 0.2
9	1.7 ± 1.2	1.1 ± 0.1
10	1.5 ± 0.2	0.38 ± 0.04

FIG. 4

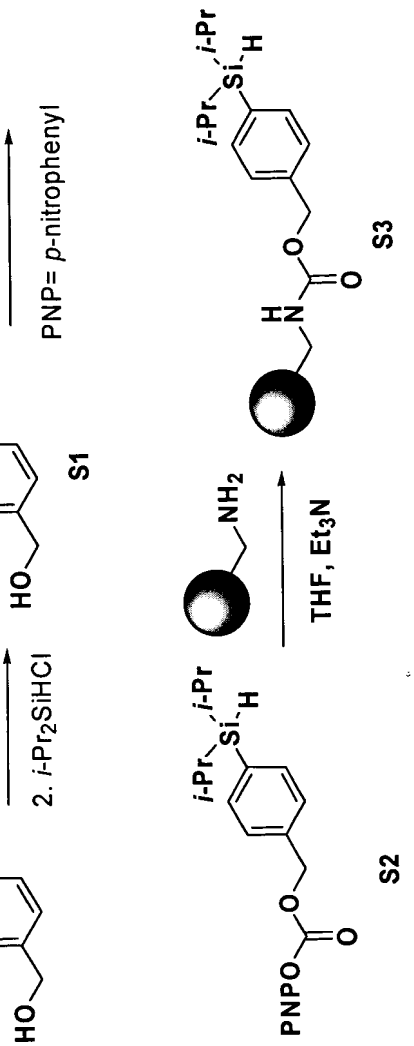

Br
1. t-BuLi
2. i-Pr2SiHCl
HO
i-Pr
i-Pr
Si
H
HO
S1
O
Cl
PNPO
PNP= p-nitrophenyl
NH2
THF, Et3N
PNPO
O
O
S2
H
N
O
O
S3

FIG. 5

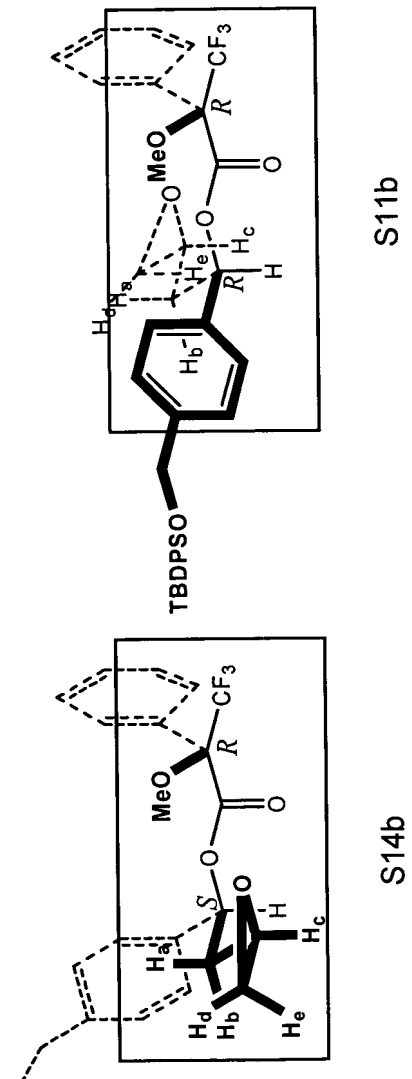

TBDPSO
MeO
CF3
R
S11b
TBDPSO
MeO
CF3
R
S14b

FIG. 6

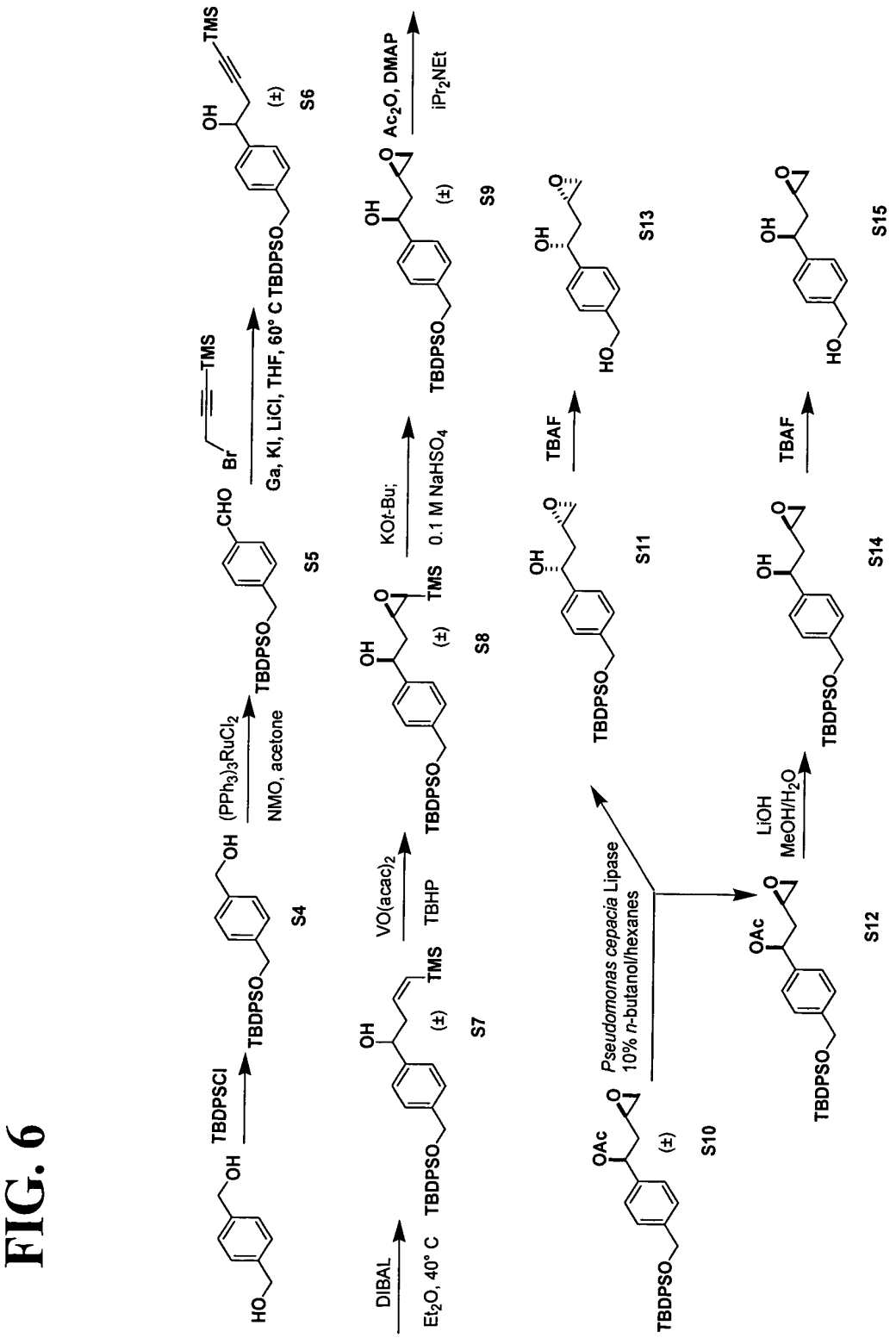

HO
OH
TBDPSCl
TBDPSO
S4
(PPh3)3RuCl2
NMO, acetone
CHO
S5
Br
TMS
Ga, KI, LiCl, THF, 60° C
OH
TMS
(±)
S6
DIBAL
Et2O, 40° C
VO(acac)2
TBHP
S7
KOt-Bu;
0.1 M NaHSO4
S8
Ac2O, DMAP
iPr2NEt
S9
Pseudomonas cepacia Lipase
10% n-butanol/hexanes
S10
TBAF
S11
S13
LiOH
MeOH/H2O
S12
S14
S15

FIG. 7

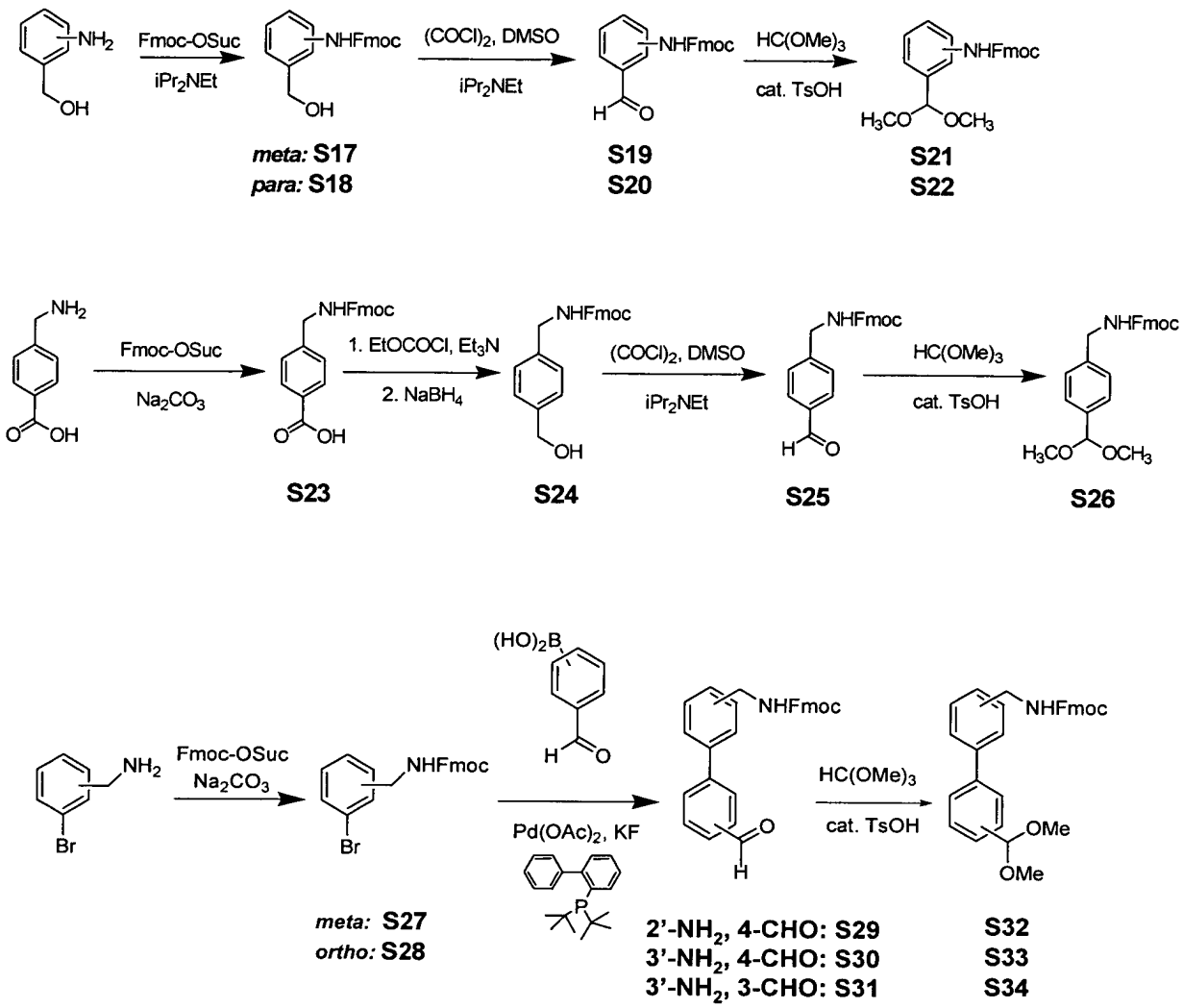

NH2
Fmoc-OSuc
iPr2NEt
NHFmoc
OH
(COCl)2, DMSO
iPr2NEt
H
O
HC(OMe)3
cat. TsOH
H3CO
OCH3
meta: S17
para: S18
S19
S20
S21
S22
Fmoc-OSuc
Na2CO3
1. EtOCOCl, Et3N
2. NaBH4
S23
S24
S25
S26
(HO)2B
Br
Pd(OAc)2, KF
P
meta: S27
ortho: S28
2'-NH2, 4-CHO: S29
3'-NH2, 4-CHO: S30
3'-NH2, 3-CHO: S31
OMe
S32
S33
S34

FIG. 8A

FIG. 8B

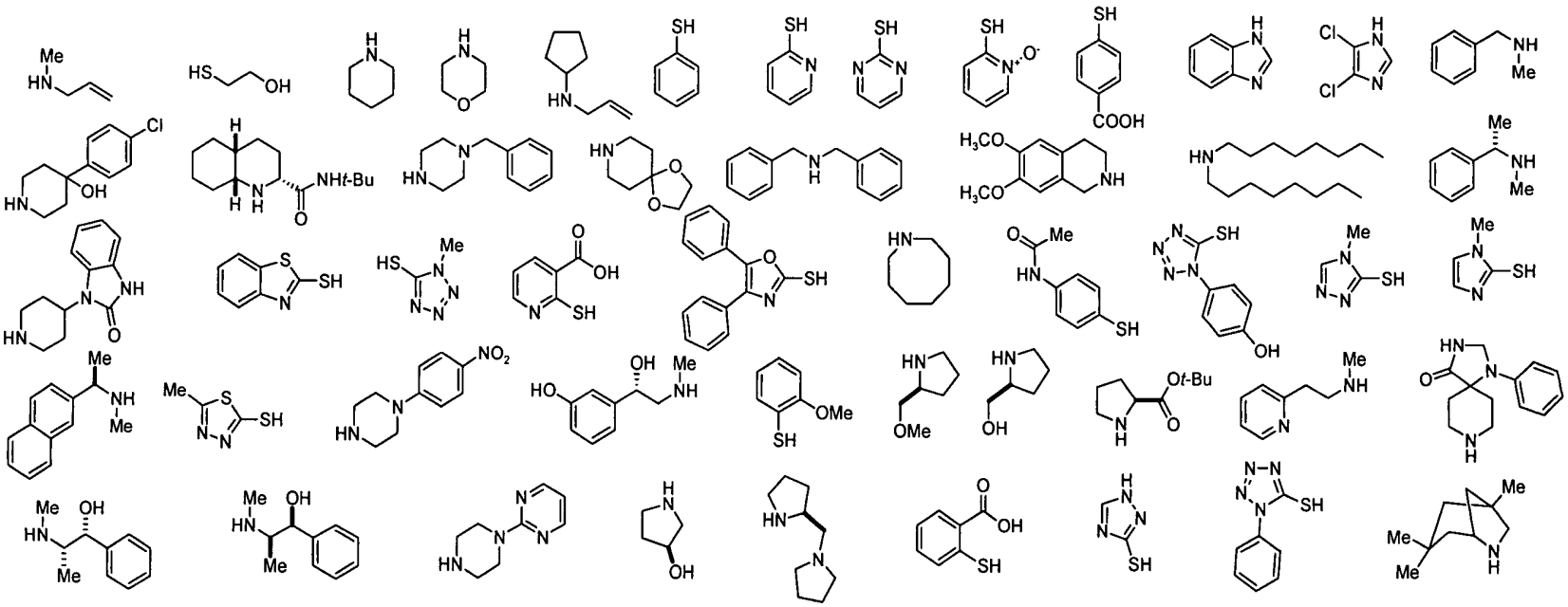


FIG. 8C

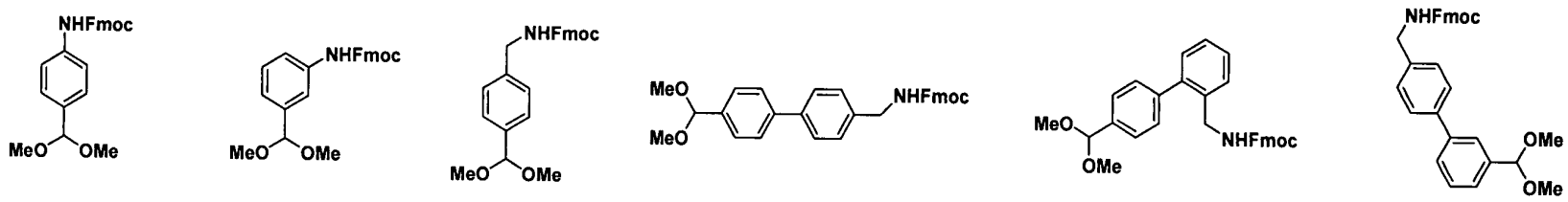

NHFmoc
MeO
OMe
NHFmoc
MeO
OMe
NHFmoc
MeO
OMe
MeO
MeO
NHFmoc
MeO
OMe
NHFmoc
NHFmoc
OMe
OMe

FIG. 8D

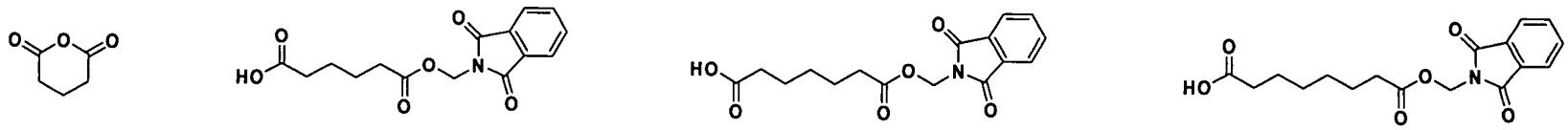

O
O
O
HO
O
O
O
N
O
O
HO
O
O
O
N
O
O
HO
O
O
O
N
O
O

FIG. 9A

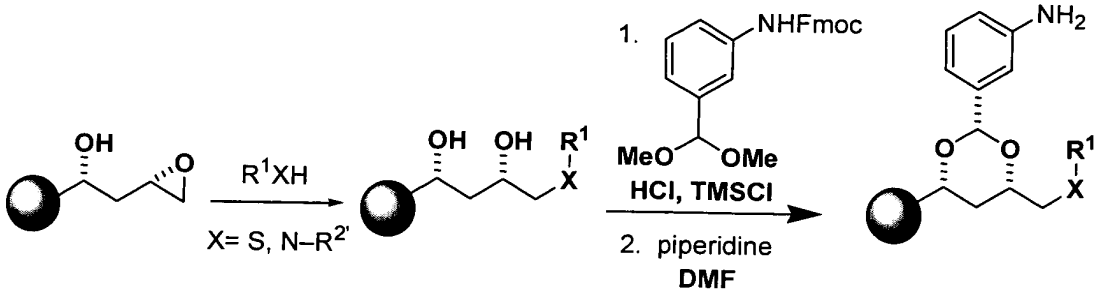


FIG. 9B

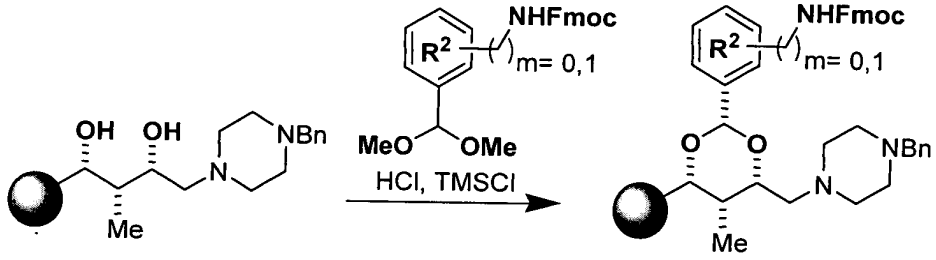


FIG. 9C

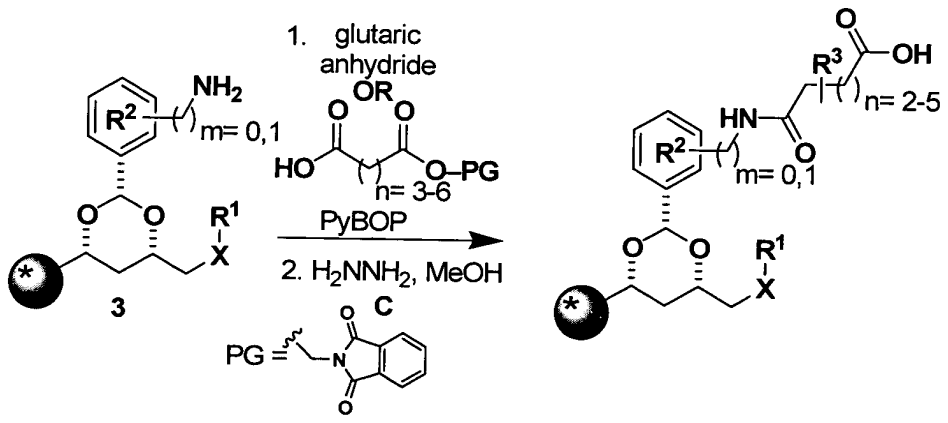
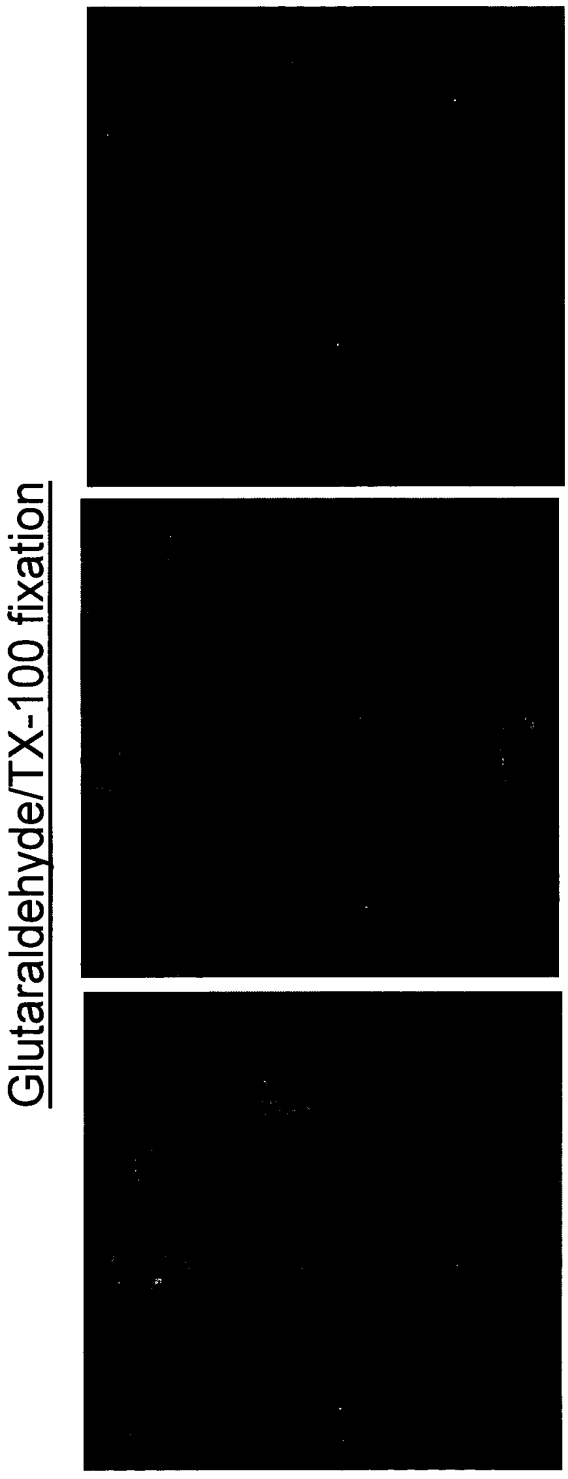
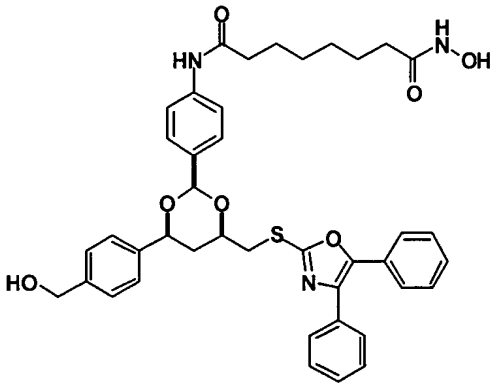


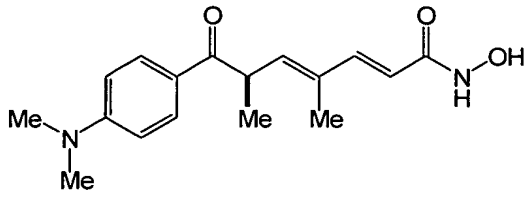
FIG. 10



Dimethylsulfoxide (0.1%)

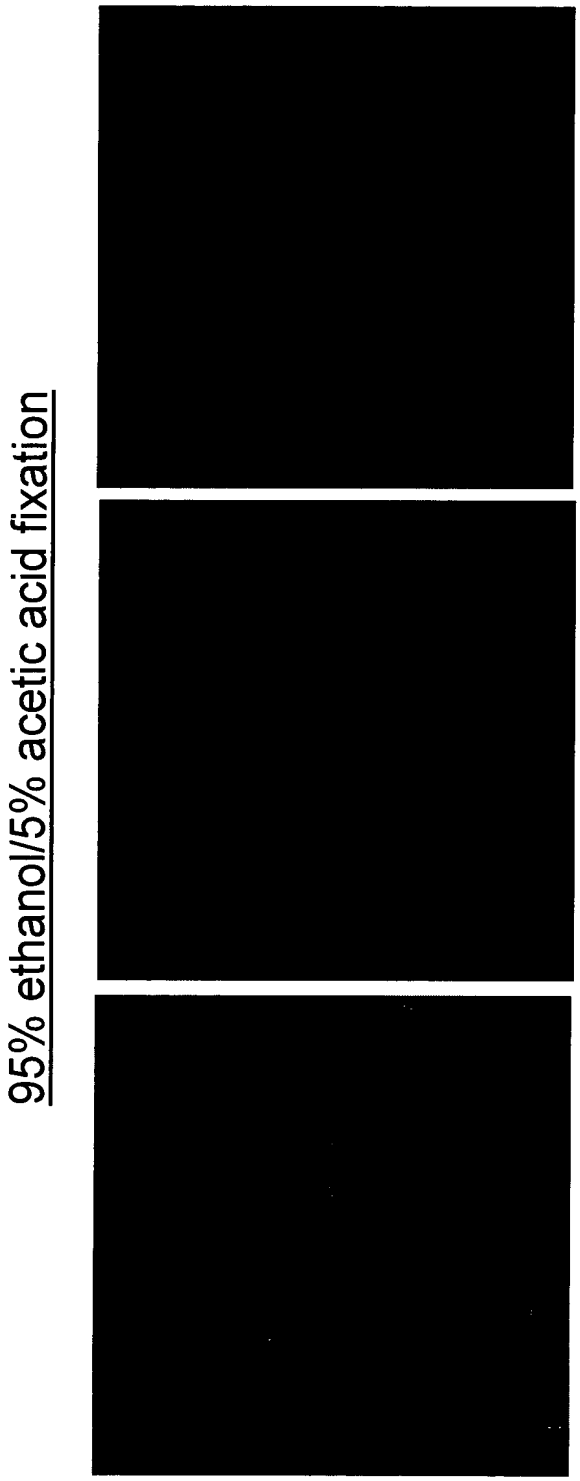


JCWII144 (200 nM)

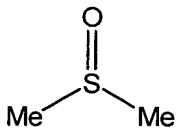


trichostatin (100 nM)

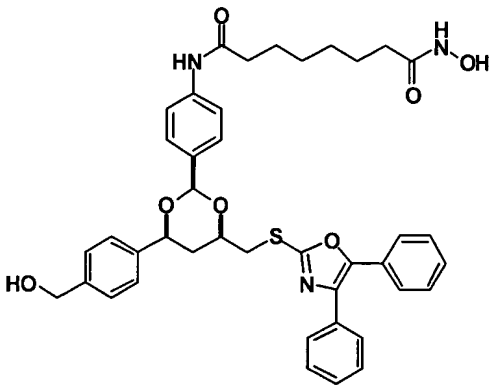
FIG. 11



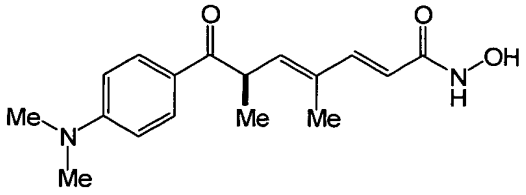
95% ethanol/5% acetic acid fixation



Dimethylsulfoxide (0.1%)

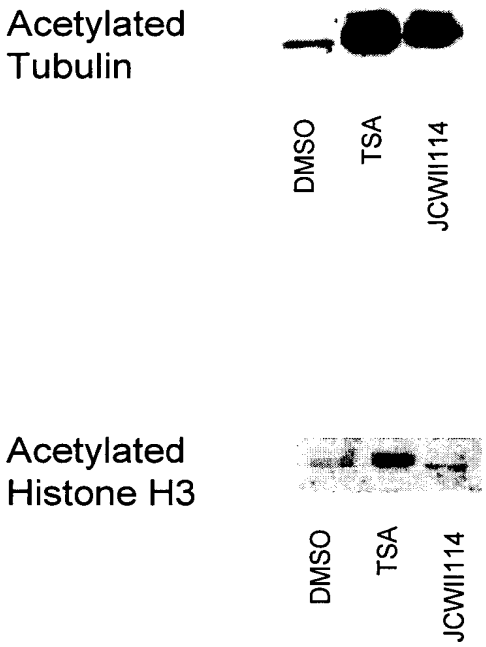


JCWII144 (2 μM)



trichostatin (1 μM)

FIG. 12



Notes:
TSA treatment at 300nM
JCWII114 treatment at 2 μM

FIG. 13A

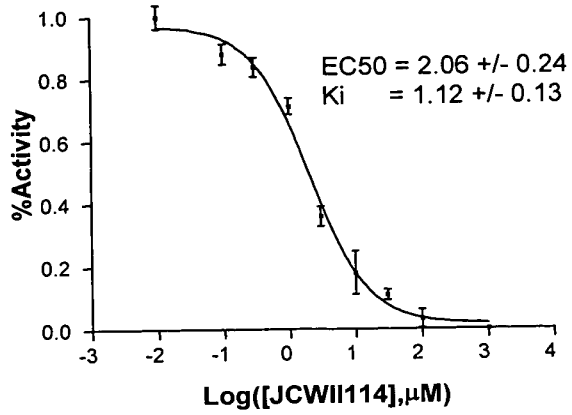


FIG. 13B

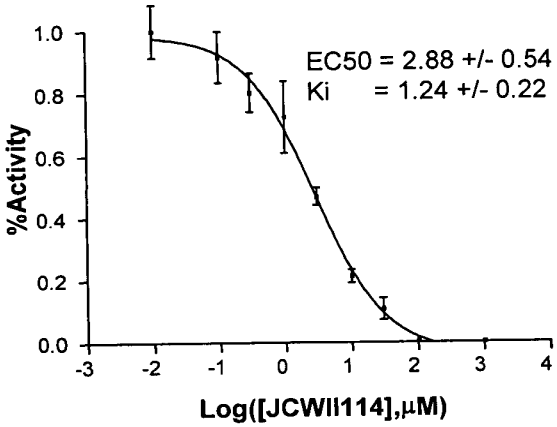


FIG. 13C

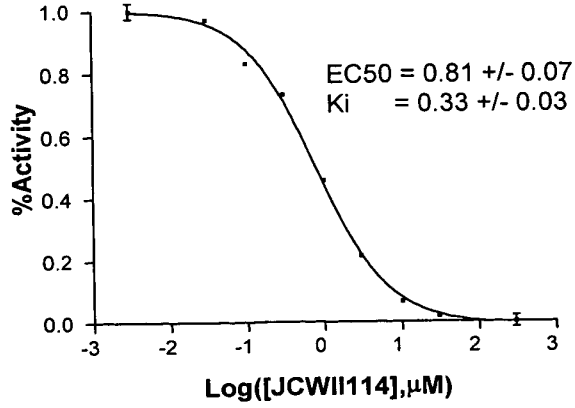


FIG. 14

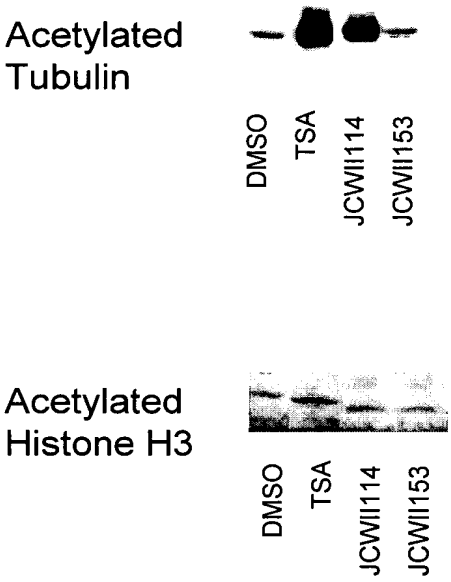

Acetylated Tubulin
DMSO
TSA
JCWII114
JCWII153
Acetylated Histone H3
DMSO
TSA
JCWII114
JCWII153

FIG. 15

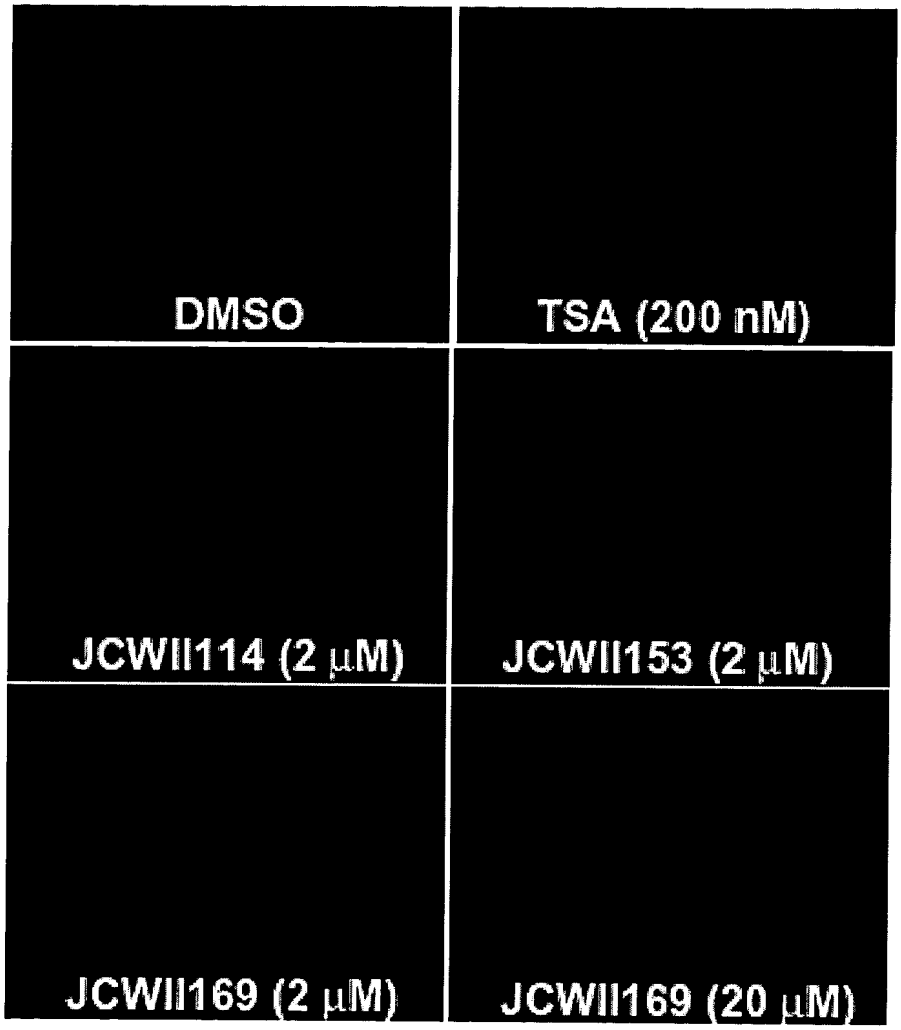

DMSO
TSA (200 nM)
JCWII114 (2 μM)
JCWII153 (2 μM)
JCWII169 (2 μM)
JCWII169 (20 μM)

FIG. 16

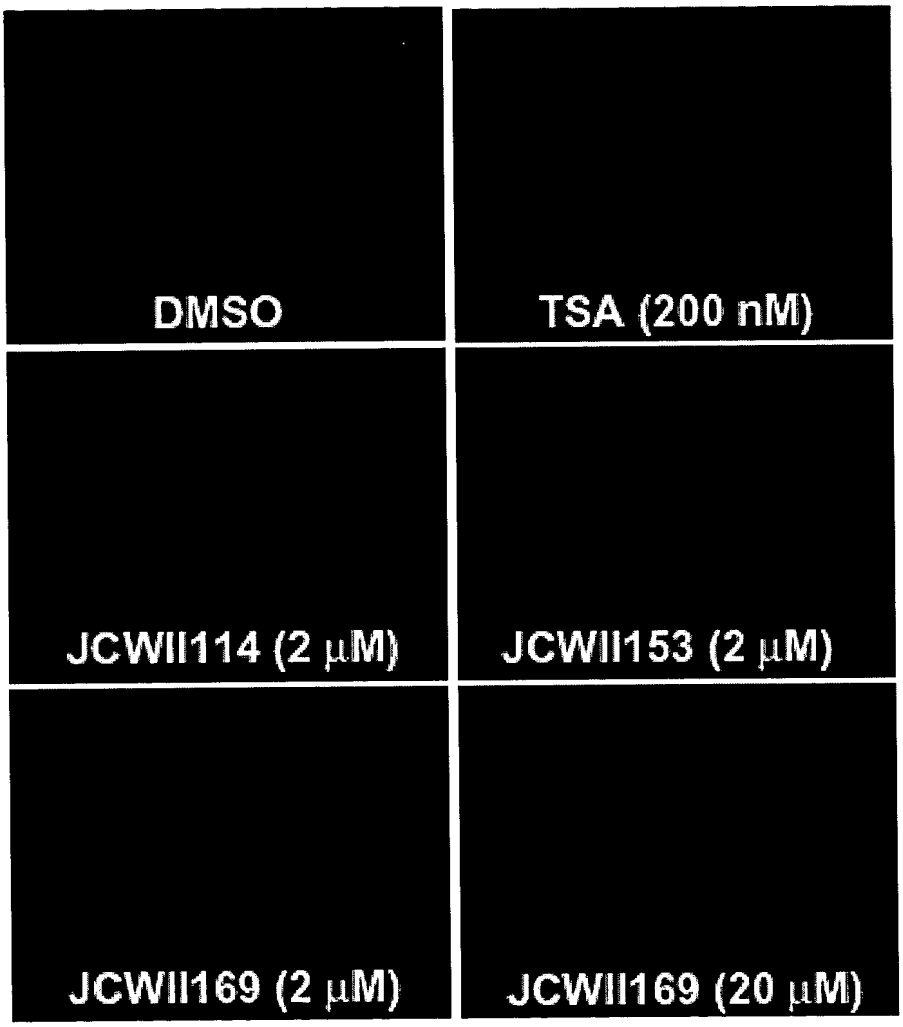

DMSO
TSA (200 nM)
JCWII114 (2 μM)
JCWII153 (2 μM)
JCWII169 (2 μM)
JCWII169 (20 μM)

FIG. 17A

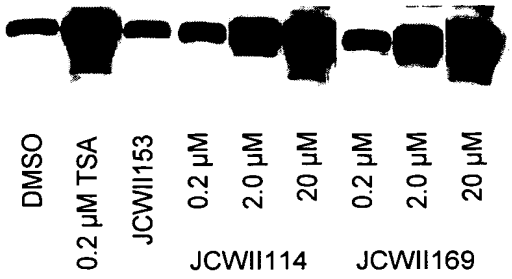


FIG. 17B

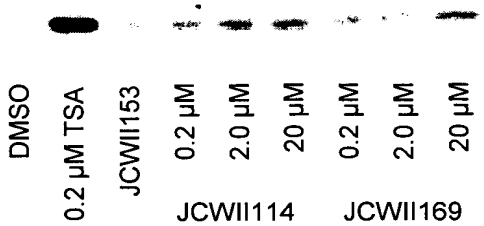


FIG. 17C

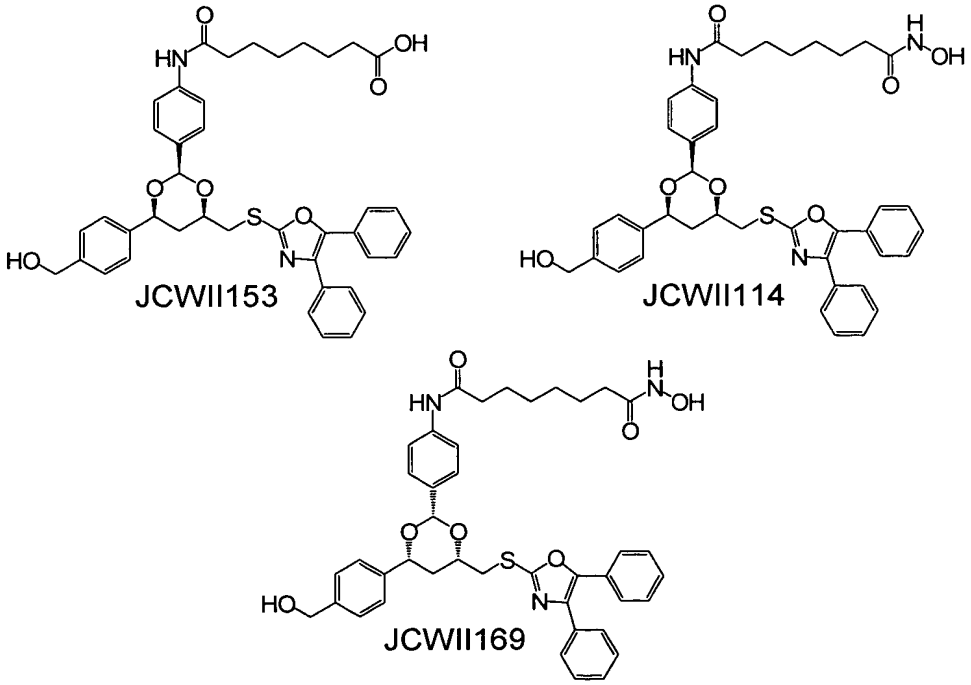


FIG. 18

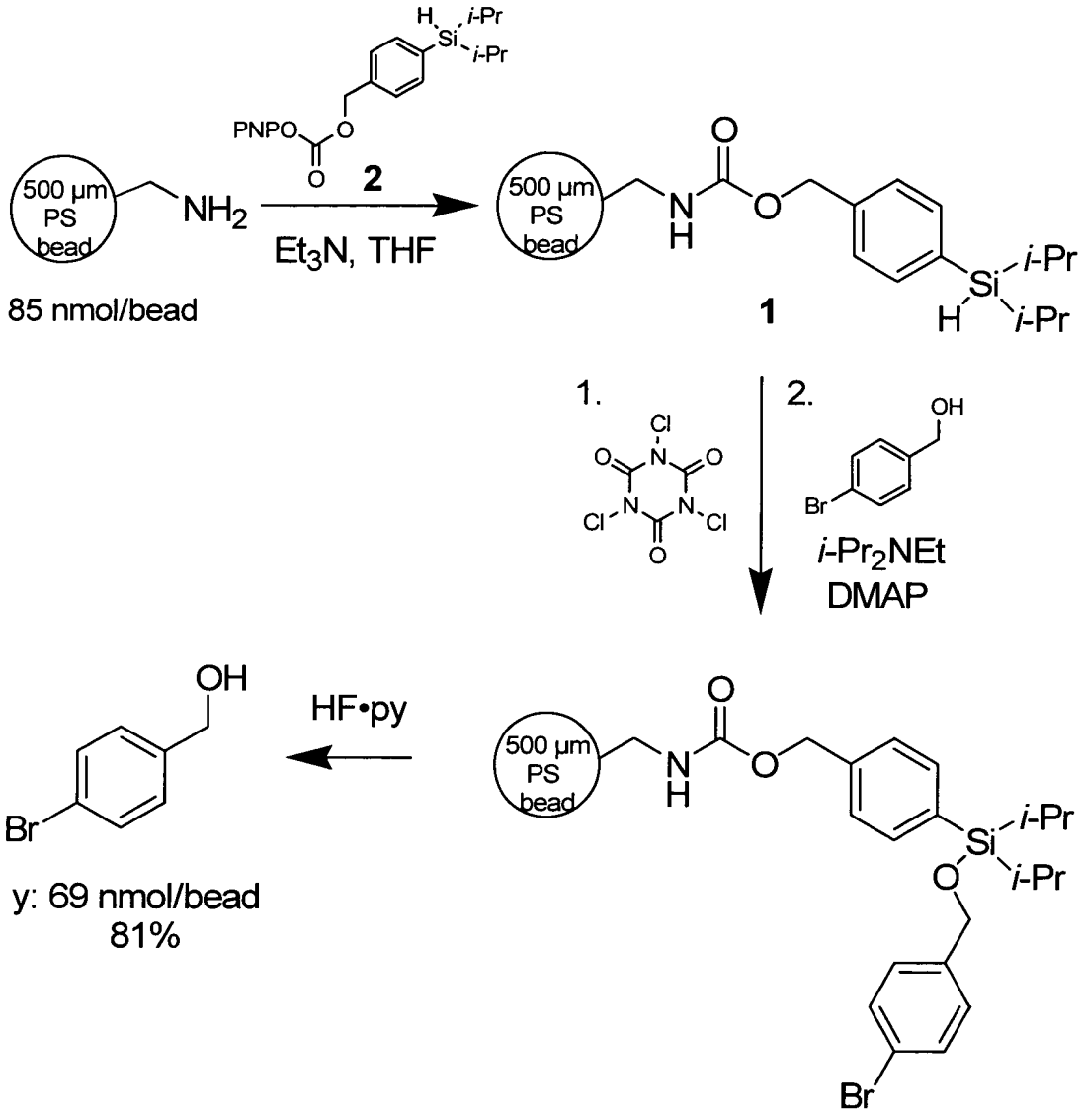

500 µm PS bead
NH2
85 nmol/bead
PNPO
O
2
Et3N, THF
1
1.
2.
i-Pr2NEt
DMAP
HF•py
y: 69 nmol/bead
81%
Br
OH
Si
i-Pr
H
N
O
Cl

FIG. 19

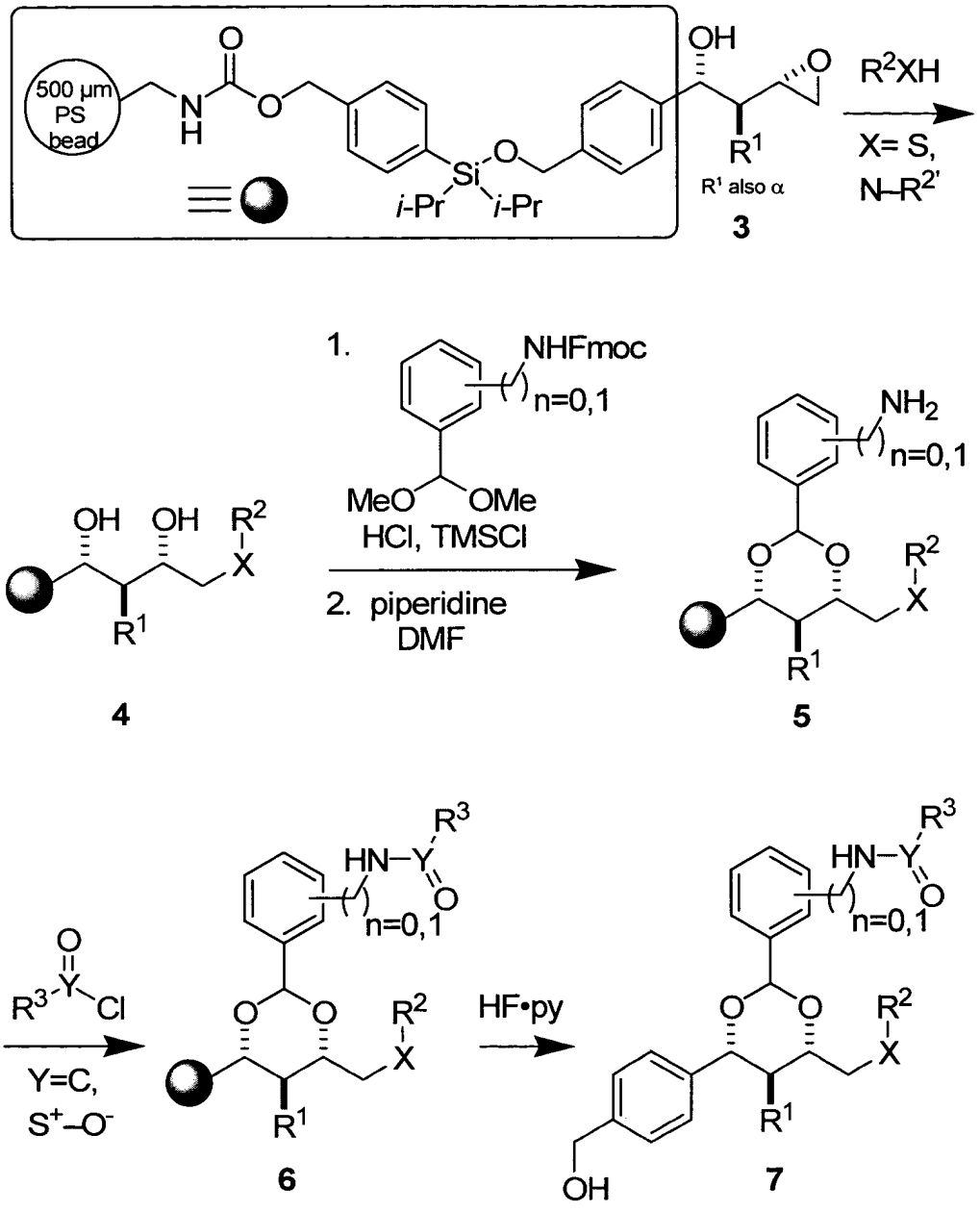

500 µm PS bead
i-Pr
i-Pr
R1 also α
3
R2XH
X= S, N–R2'
1.
NHFmoc
n=0,1
MeO
OMe
HCl, TMSCl
2. piperidine
DMF
4
5
NH2
n=0,1
R3
HN–Y
n=0,1
R3–Y–Cl
Y=C, S+–O-
6
HF•py
7
OH

FIG. 20A

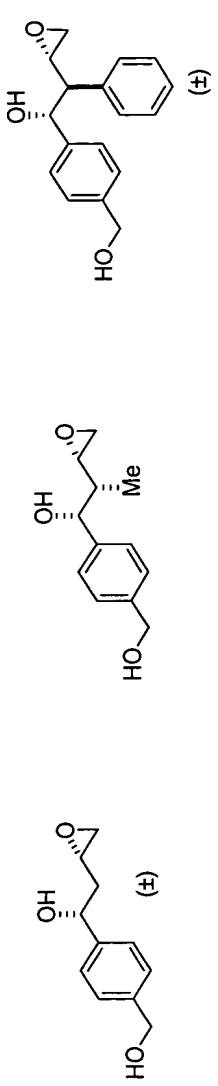


FIG. 20B

FIG. 20C

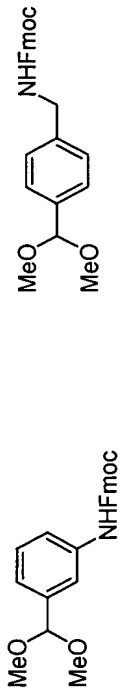


FIG. 20D

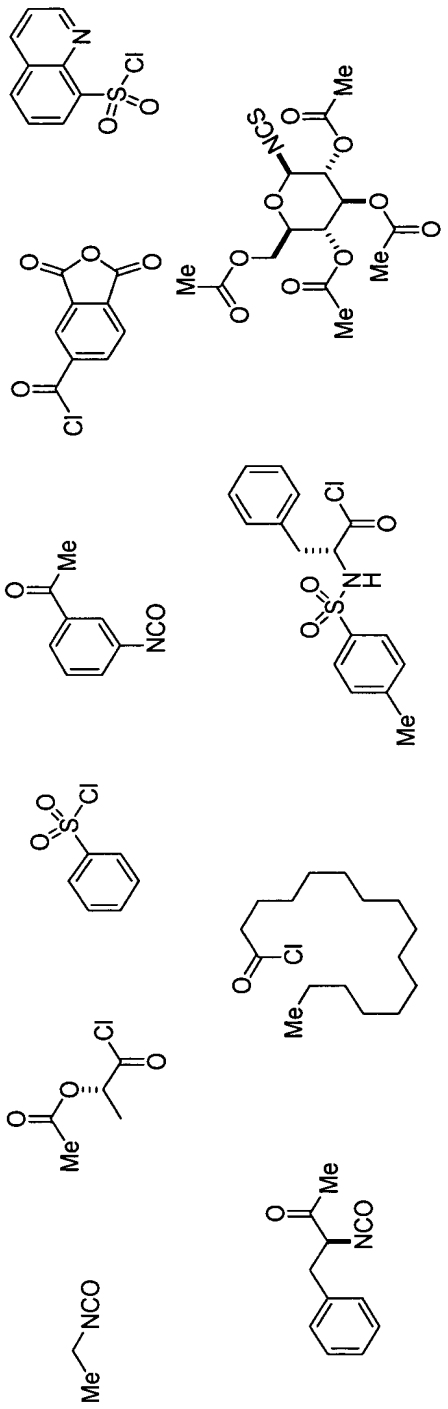
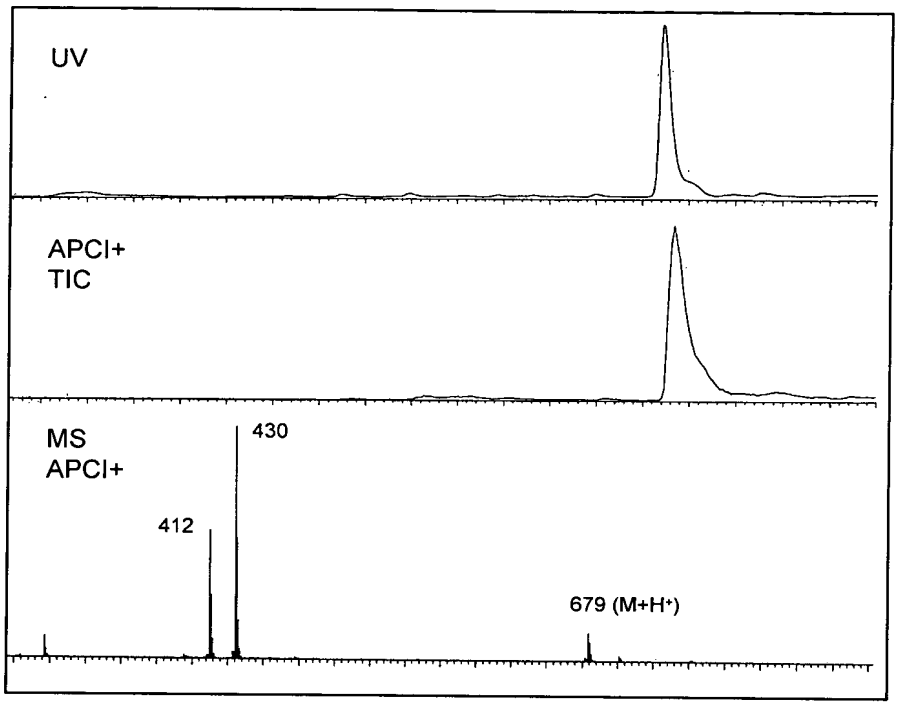


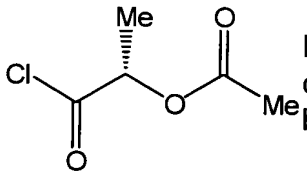
FIG. 21A



To FIG. 21B

FIG. 21B

Final building block



Mass contributed 115

Mass of amine precursor 678-115 +1=564

To FIG. 21C

FIG. 21C

acetal fragment 103	3-epoxy alcohols		
	194	**208**	**270**
71	368	382	444
78	375	389	451
87	384	398	460
101	398	412	474
101	398	412	474
111	408	422	484
113	410	424	486
116	413	427	489
118	415	429	491
125	422	436	498
126	423	437	499
127	424	438	500
132	429	443	505
136	433	447	509
136	433	447	509
143	440	454	516
154	451	465	527
155	452	466	528
164	461	475	537
165	462	476	538
167	464	478	540
167	464	478	540
193	490	504	566
194	491	505	567
211	508	**522**	584
217	514	528	590
221	518	532	594
231	528	542	604
253	550	564	626
263	560	574	636

acetal fragment 117	3-epoxy alcohols		
	194	**208**	**270**
71	382	396	458
78	389	403	465
87	398	412	474
101	412	426	488
101	412	426	488
111	422	436	498
113	424	438	500
116	427	441	503
118	429	443	505
125	436	450	512
126	437	451	513
127	438	452	514
132	443	457	519
136	447	461	523
136	447	461	523
143	454	468	530
154	465	479	541
155	466	480	542
164	475	489	551
165	476	490	552
167	478	492	554
167	478	492	554
193	504	518	580
194	505	519	581
211	**522**	536	598
217	528	542	604
221	532	546	608
231	542	556	618
253	564	578	640
263	574	588	650

30 nucleophiles

To FIG. 21D

FIG. 21D

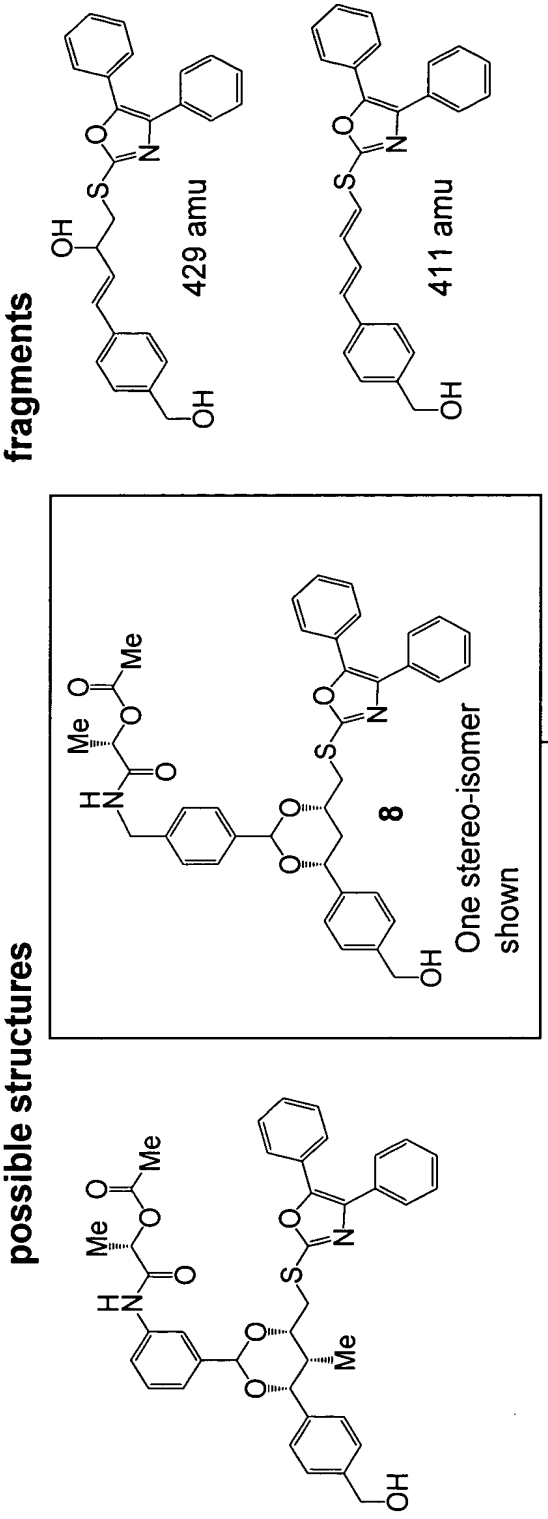


FIG. 21E

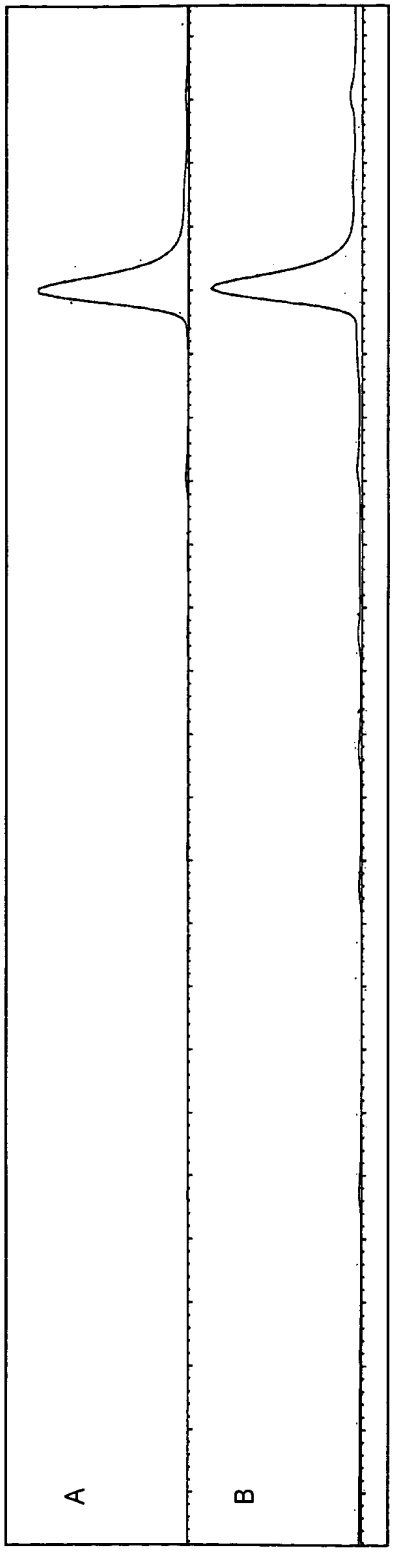
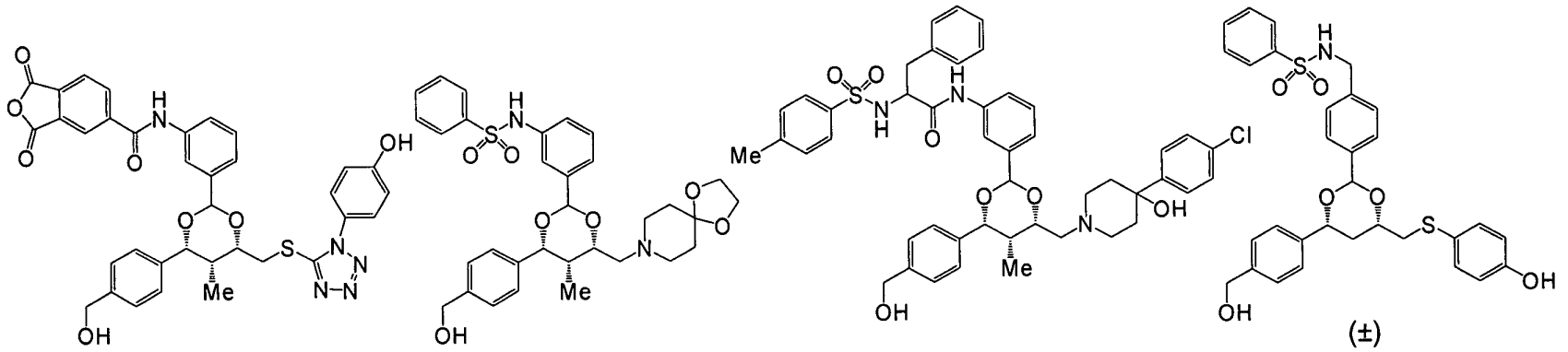


FIG. 22A



9

Cyclin B degradation
In Xenopus extract

10

Cyclin B degradation
In HeLa cells

11

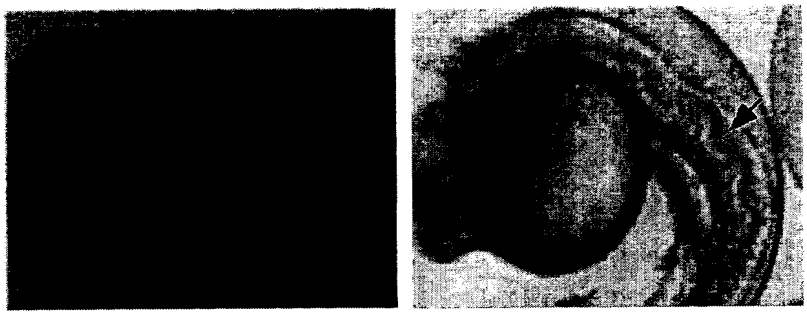
Detached
HeLa cells

(±)

12

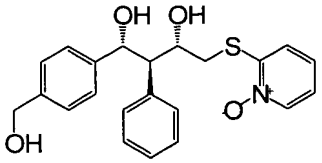
Spherical actin
Staining phenotype

FIG. 22B



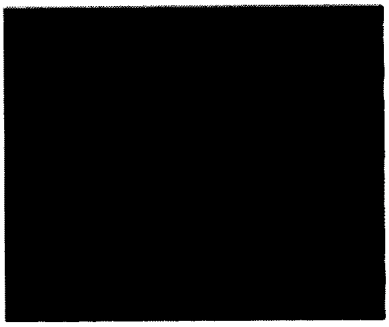
No compound

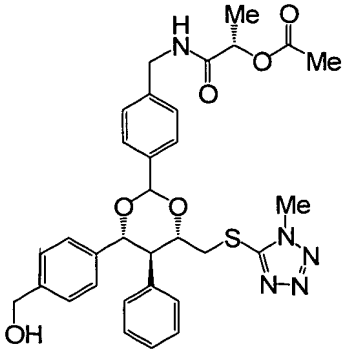
60 μM
24h



(±)
13

FIG. 22C





14

One stereoisomer shown

FIG. 23A

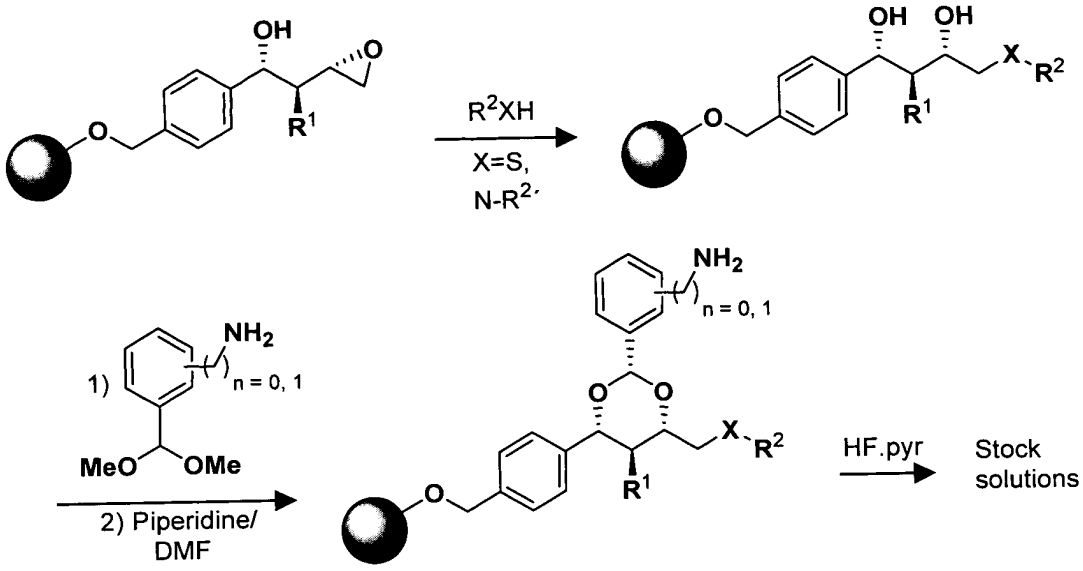


FIG. 23B

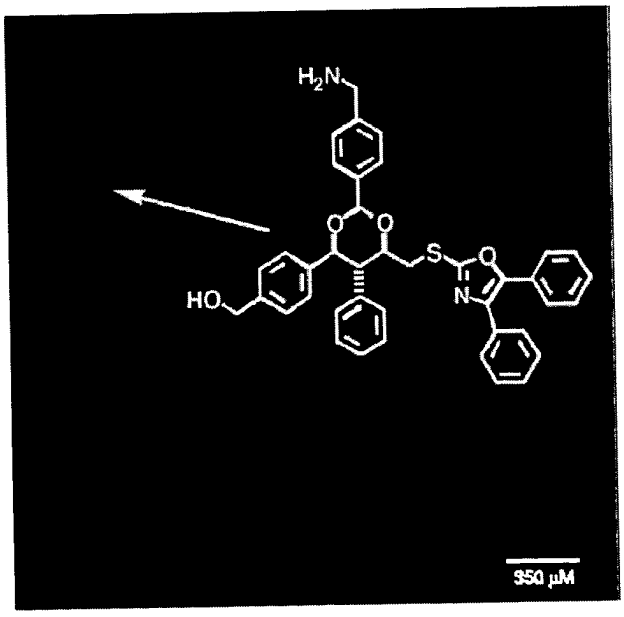


FIG. 24A

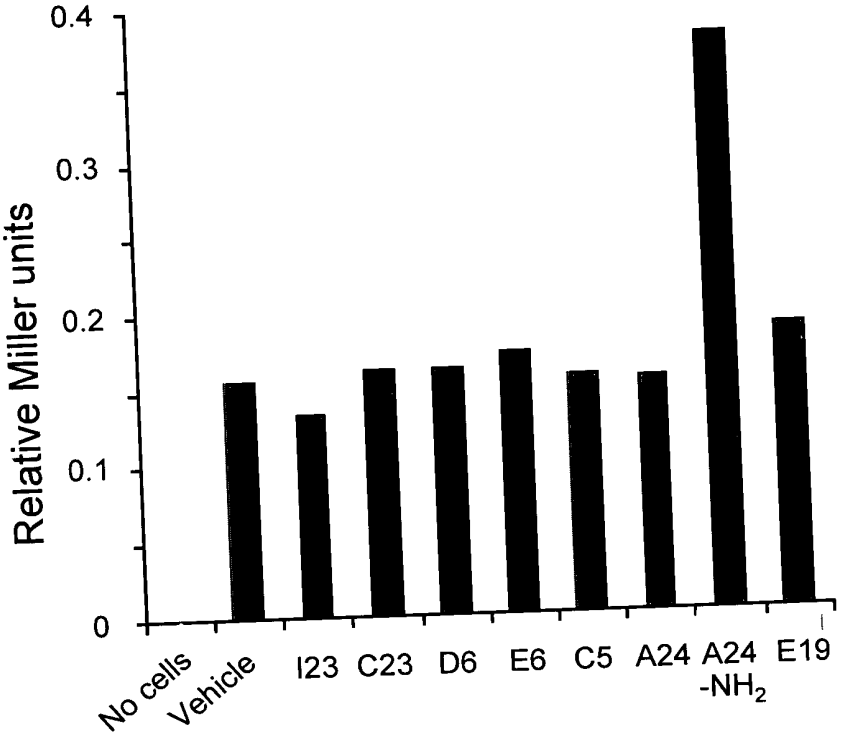


FIG. 24B

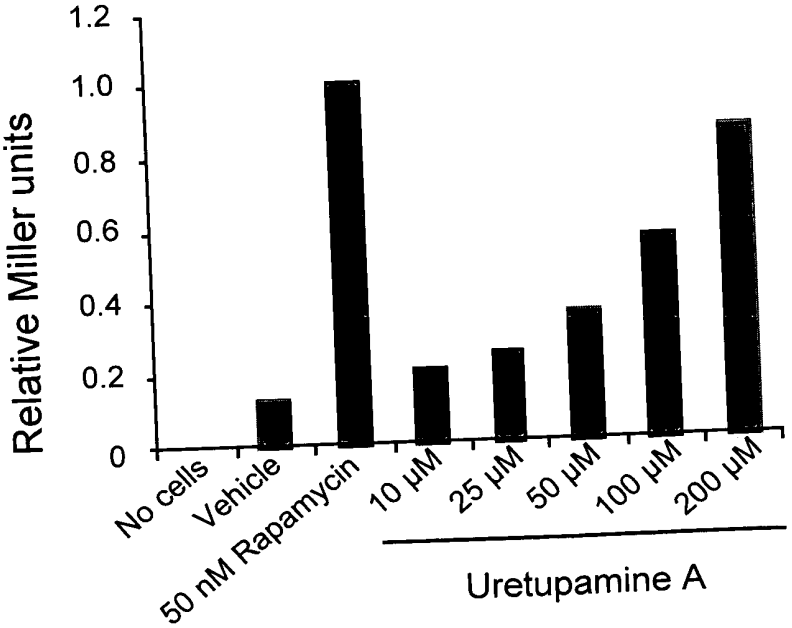
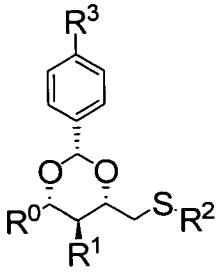


FIG. 24C



Uretupamine	R^0	R^1	R^2	R^2	Activity
A	$HOCH_2$-4-Ph	Ph	2-MDPO	CH_2NH_2	56
B	$HOCH_2$-4-Ph	H	2-MDPO	CH_2NH_2	105
C	$HOCH_2$-4-Ph	(β)-CH_3	2-MDPO	CH_2NH_2	41*
D	$HOCH_2$-4-Ph	H	Ph	CH_2NH_2	7
E	$HOCH_2$-4-Ph	H	2-MBO	CH_2NH_2	10
F	$HOCH_2$-4-Ph	H	2-MDPO	H	14
G	$HOCH_2$-4-Ph	H	2-MDPO	CH_2NHAc	16
H	H	H	2-MDPO	CH_2NH_2	13

FIG. 24D

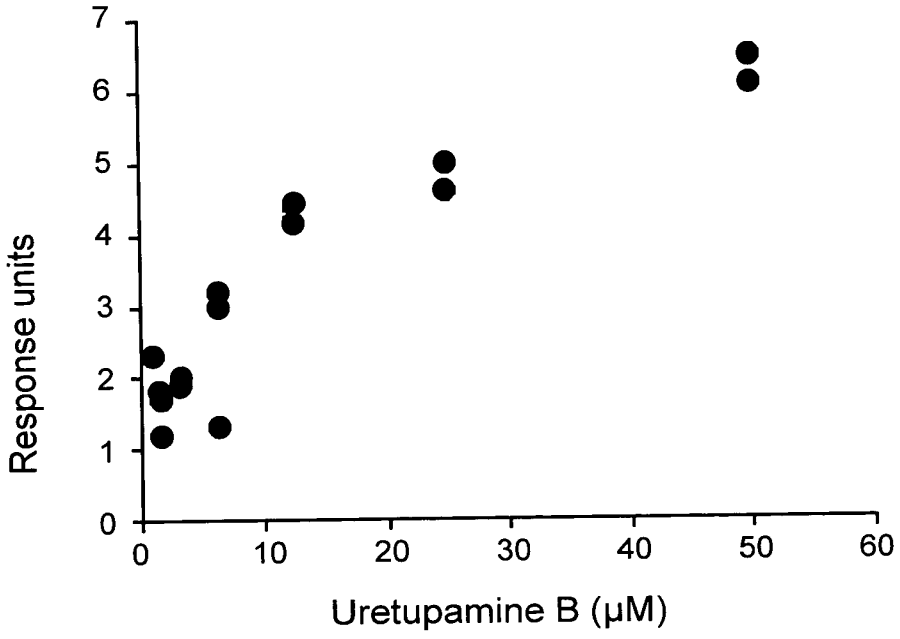
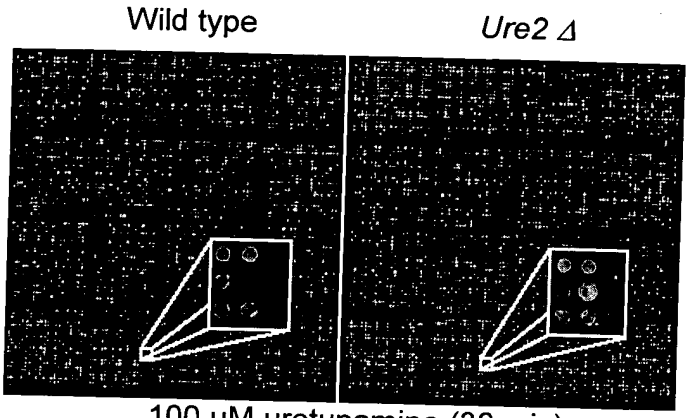


FIG. 25A



	w.t.	*Ure2 Δ*
PUT1	+4.0	+1.1
PUT2	+2.3	+1.1
UGA1	+2.2	+1.2
NIL1	+3.8	+1.1
PRB1	+4.0	+1.7

FIG. 25B

Gene sets	w.t.	*gln3Δ*	*nil1Δ*	*ure2Δ*
GAP1, MEP2, DAL5, BAT2, AGP1	+1.1	+1.1	-1.0	-1.0
PUT1, PUT2, UGA1, NIL1, PRB1	+2.3	+2.5	+1.6	+1.2

FIG. 25C

Gene sets	w.t.	*gln3Δ*	*nil1Δ*	*ure2Δ*
Whole genome	100%	89%	56%	52%
URE2-dependent genes	100%	115%	51%	59%

FIG. 26A

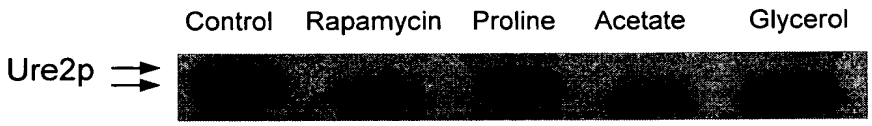


FIG. 26B

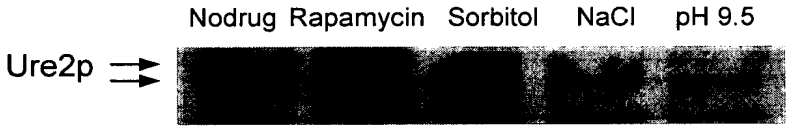


FIG. 26C

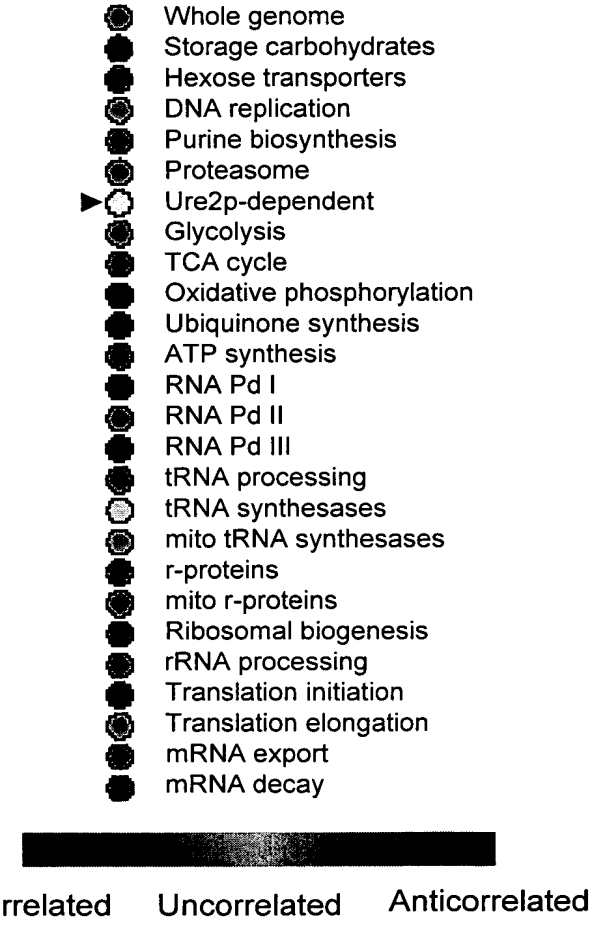


FIG. 26D

	Ethanol	Acetate
PUT1	+11.5	> +10.0
PUT2	+2.8	+1.6
UGA1	+4.7	+3.3
NIL1	+2.6	+2.5
PRB1	+2.3	+1.4
GAP1	+3.3	-9.0
DAL1	+1.7	-4.8
DAL2	+3.1	-1.5
DAL3	+2.1	< -10.0
CAR1	+2.6	-1.4

FIG. 26E

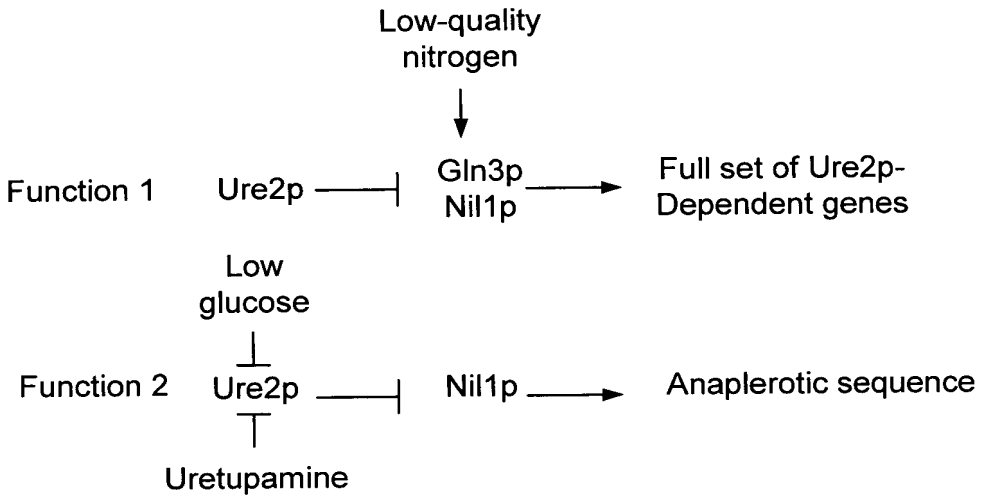


FIG. 27A

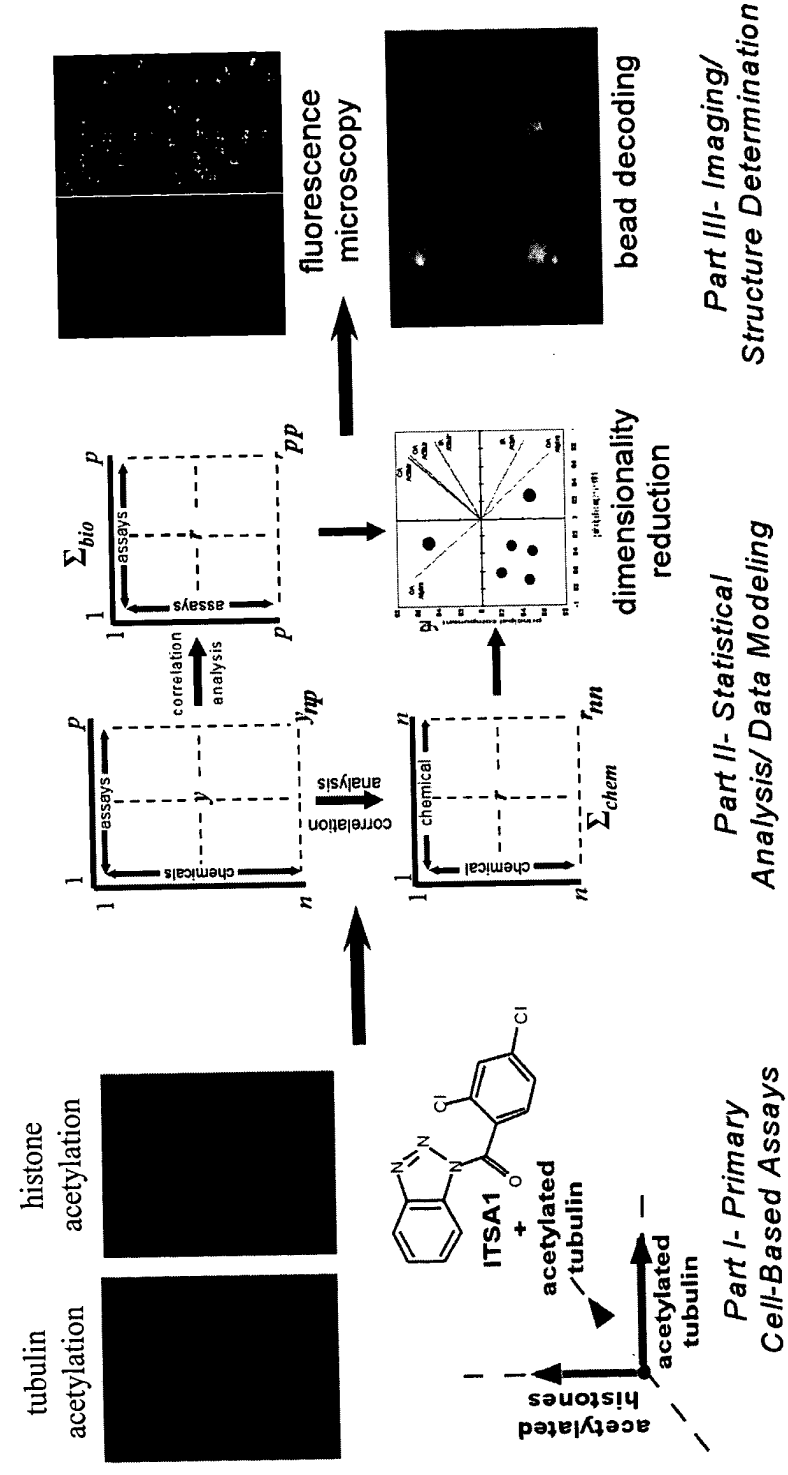
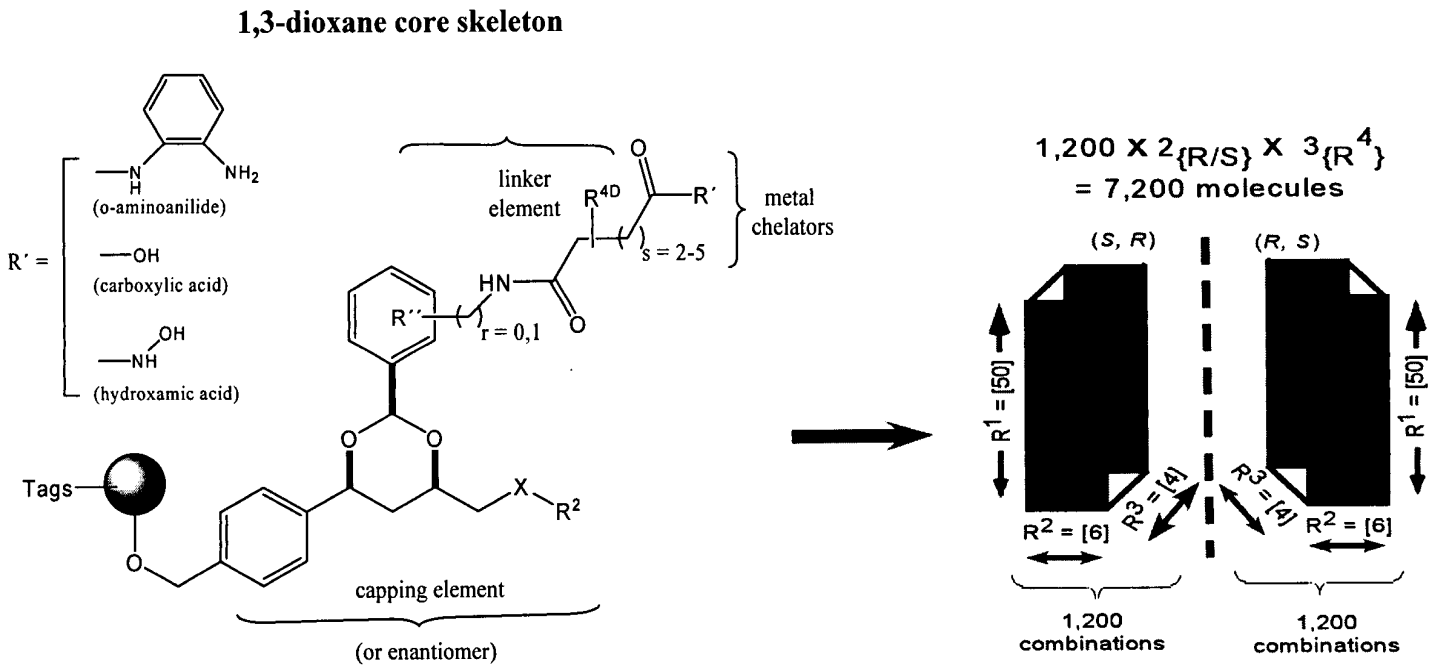

tubulin acetylation
histone acetylation
acetylated histones
acetylated tubulin
ITSA1 + acetylated tubulin
Part I- Primary Cell-Based Assays
correlation analysis
correlation analysis
chemicals
assays
chemical
assays
dimensionality reduction
Part II- Statistical Analysis/ Data Modeling
fluorescence microscopy
bead decoding
Part III- Imaging/ Structure Determination

FIG. 27B



Biasing elements in diversity-oriented synthesis

FIG. 27C

	Assay	Abbreviation	Compounds screened in duplicate
1.	Acetylated tubulin	AcTubulin	7,392
2.	Acetylated tubulin + ITSA1 (chemical genetic modifier)	ITSA1+AcTubulin	2,464 hydroxamic acids
3.	Acetylated lysine	AcLysine	7,392
4.	Acetylated histone H3	AcHistH3	2,464 hydroxamic acids
5.	Acetylated histone H4	AcHistH4	2,464 hydroxamic acids

Table 1.Summary of chemical genetic screens

FIG. 28A

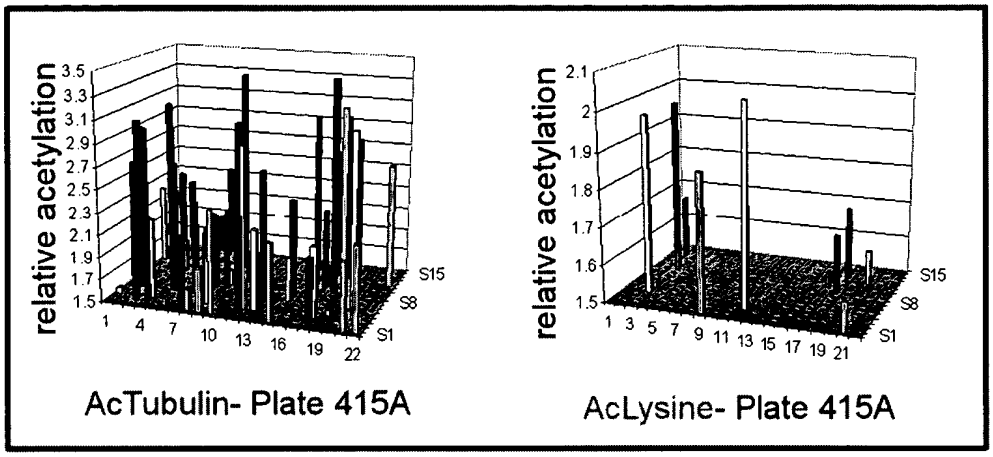


FIG. 28B

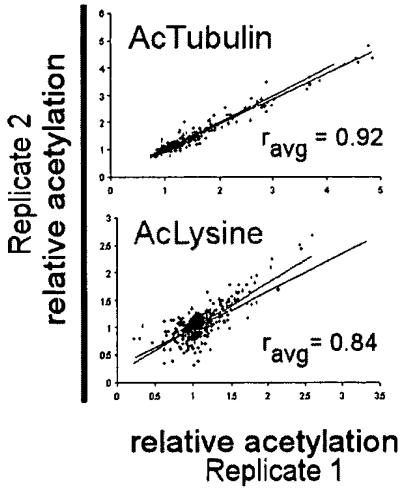


FIG. 28C

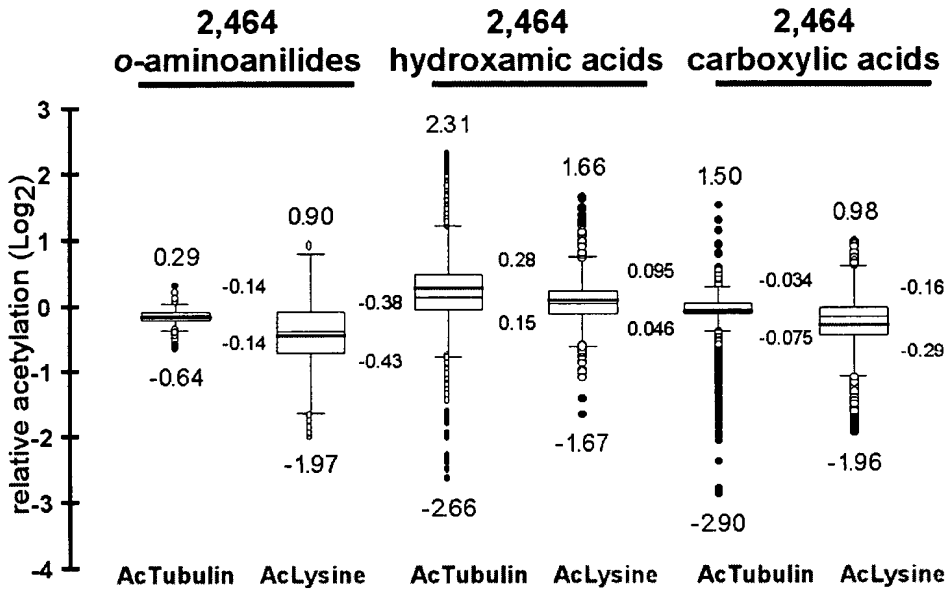
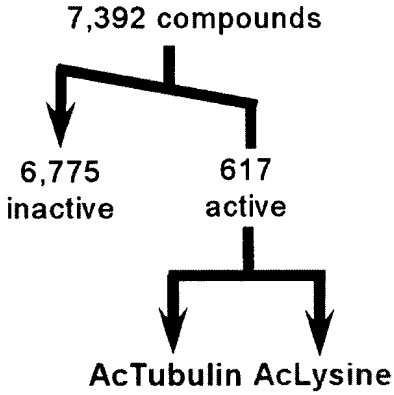


FIG. 28D



	AcTubulin	AcLysine
orthoaminoanilide	0% (n=0)	0.18% (n=13)
hydroxamic acid	6.35% (n=469)	3.5% (n=257)
carboxylic acid	0.08% (n=6)	1.0% (n=74)

FIG. 29A

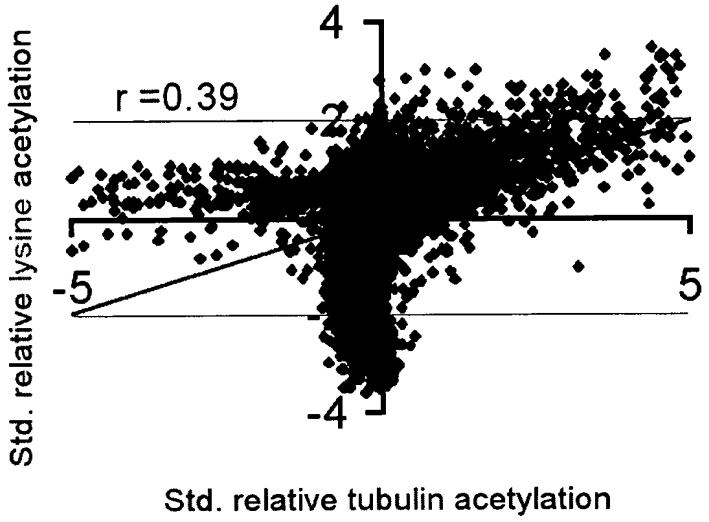


FIG. 29B

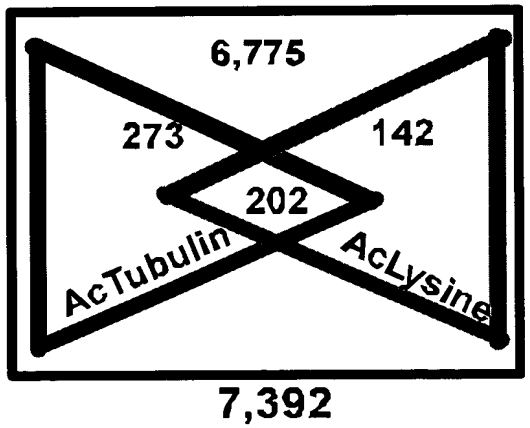


FIG. 29C

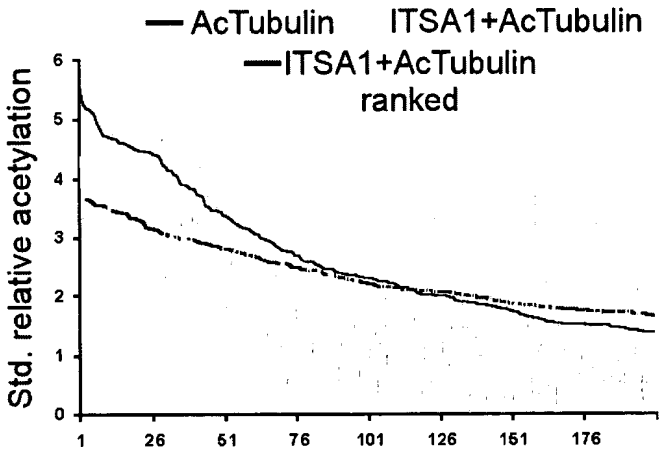


FIG. 29D

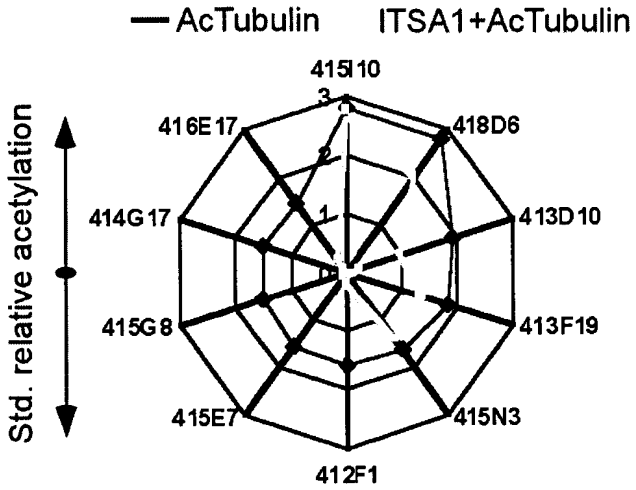


FIG. 30A

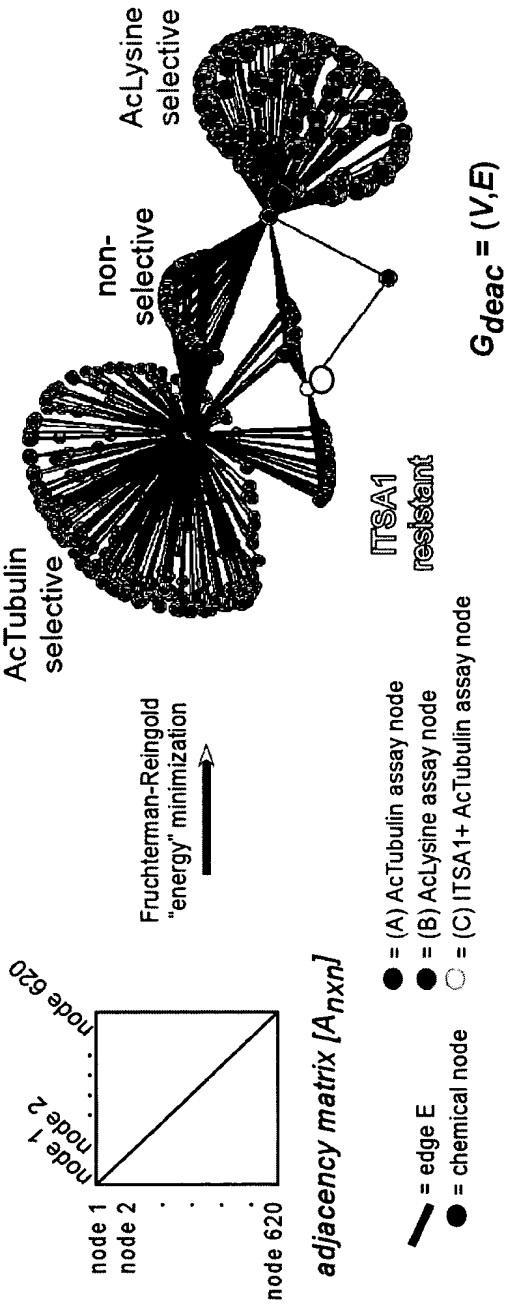

AcTubulin selective
non-selective
AcLysine selective
ITSA1 resistant
Fruchterman-Reingold "energy" minimization
node 1
node 2
node 620
adjacency matrix [A_{nxn}]
= (A) AcTubulin assay node
= (B) AcLysine assay node
= (C) ITSA1+ AcTubulin assay node
= edge E
= chemical node
$G_{deac} = (V,E)$

FIG. 30B

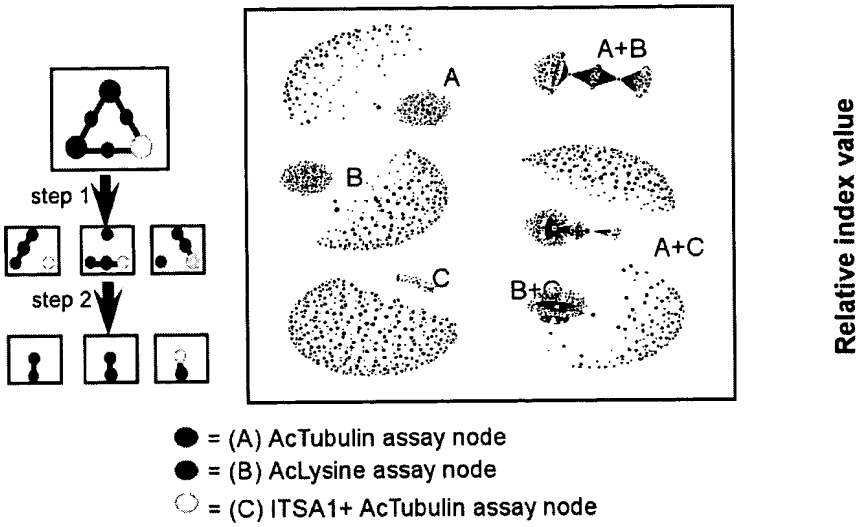


FIG. 30C

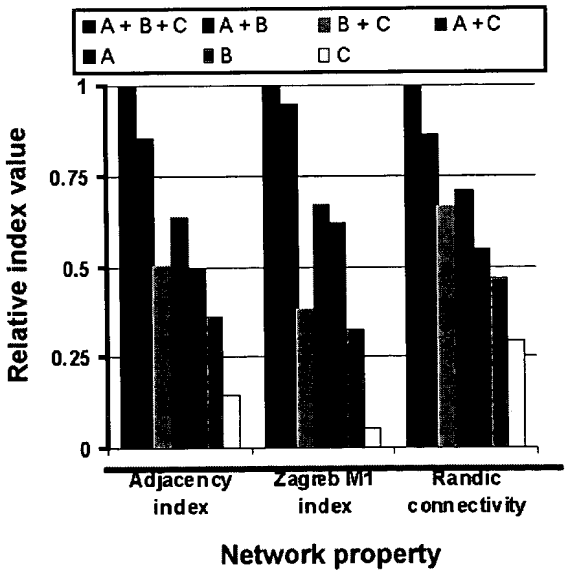
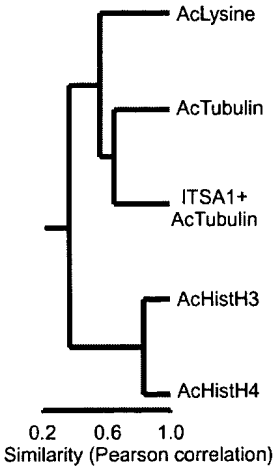
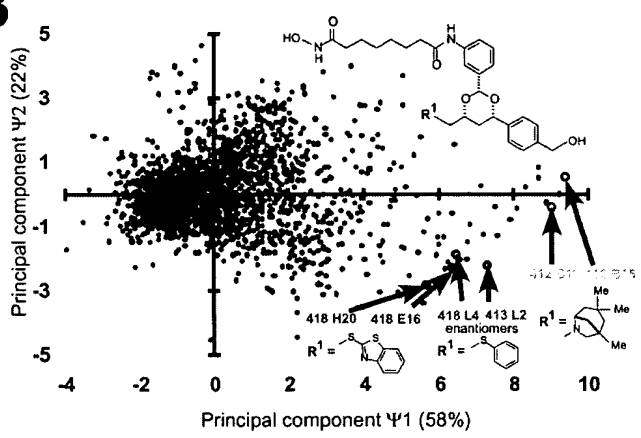


FIG. 31

A



B



C

	λ1	λ2	λ3	λ4	λ5
Eigenvalue	2.92	1.11	0.51	0.30	0.16
variance %	58.3	22.1	10.3	6.0	3.3
cumulated %	58.3	80.4	90.7	96.7	100.0

Eigenvalues

	Ψ1	Ψ2	Ψ3	Ψ4	Ψ5
AcTubulin	20.5	20.4	0.3	57.8	1.0
AcLysine	18.9	11.4	52.9	16.7	0.1
ITSA1+AcTubulin	20.1	9.3	46.7	23.4	0.5
AcHist3	19.9	30.6	0.0	0.1	49.4
AcHist4	20.6	28.3	0.1	2.0	49.0

Contribution of variables to principal components Ψ1 - Ψ5

FIG. 32

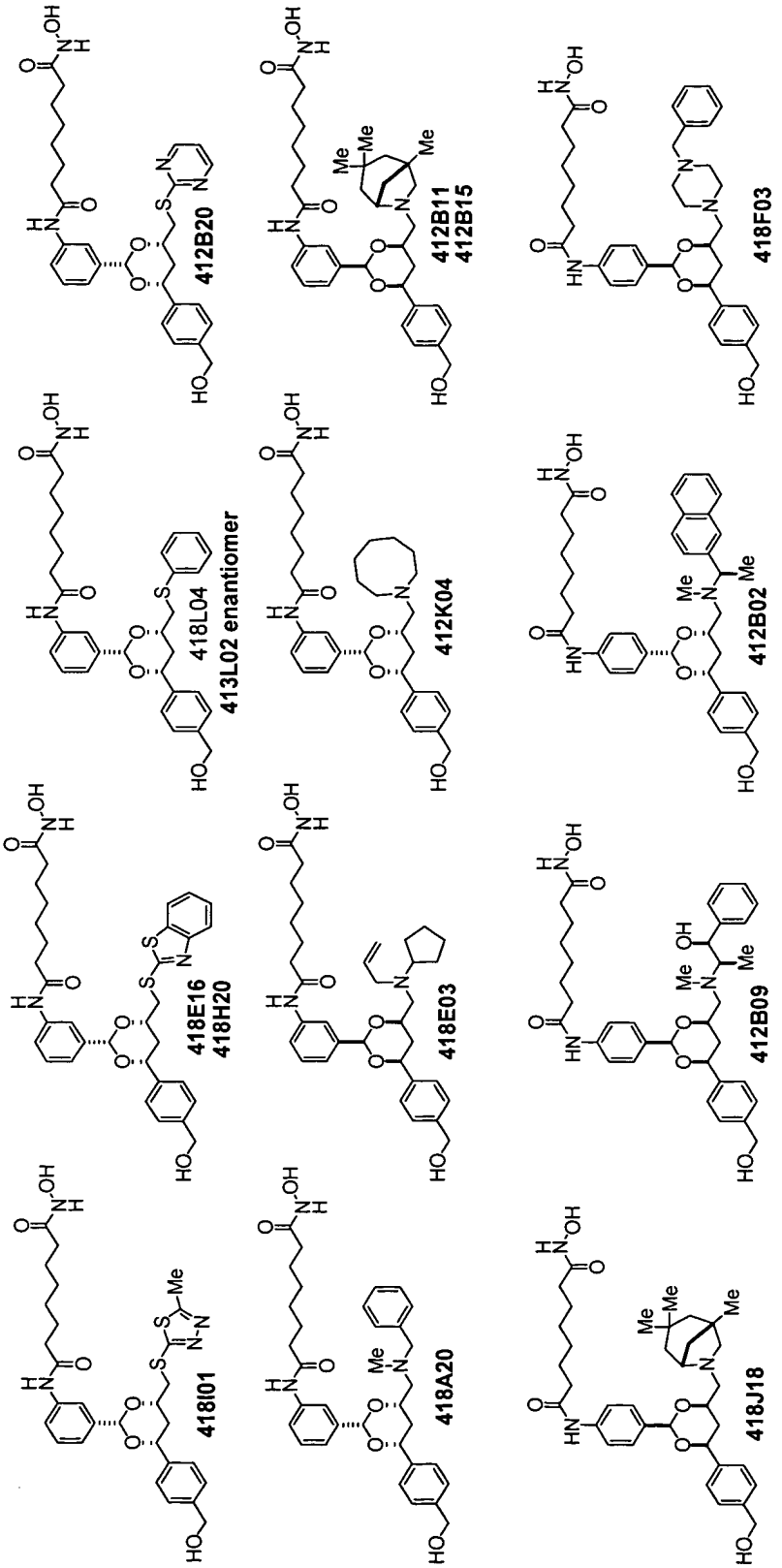
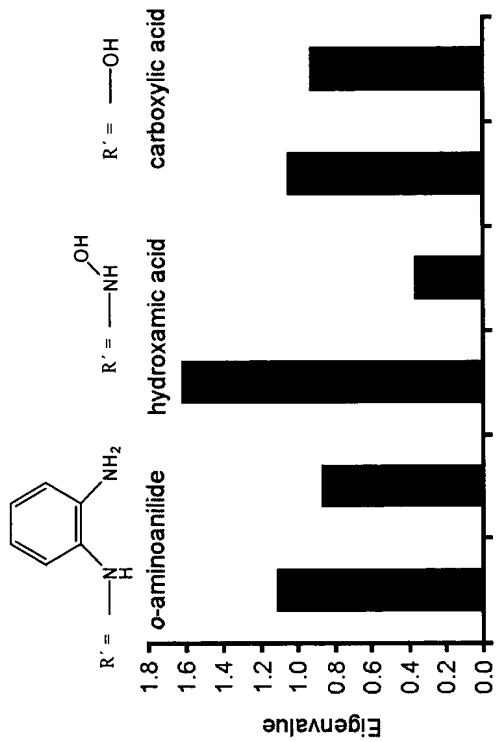

418I01
418E16
418H20
413L02 enantiomer
418L04
412B20
418A20
418E03
412K04
412B11
412B15
418J18
412B09
412B02
418F03

FIG. 33A



Eigenvalue	1.12	0.88	1.63	0.37	1.06	0.94
variance %	56.0	44.0	81.5	18.5	53.0	47.0
cumulated %	56.0	100.0	81.5	100.0	53.0	100.0

FIG. 33B

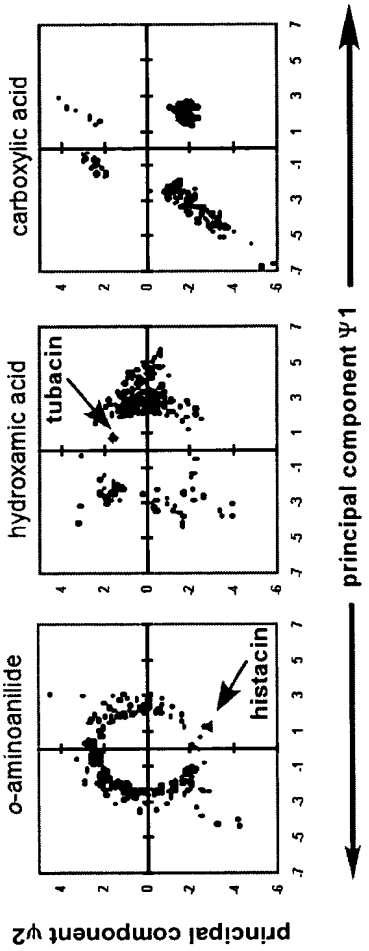


FIG. 33C

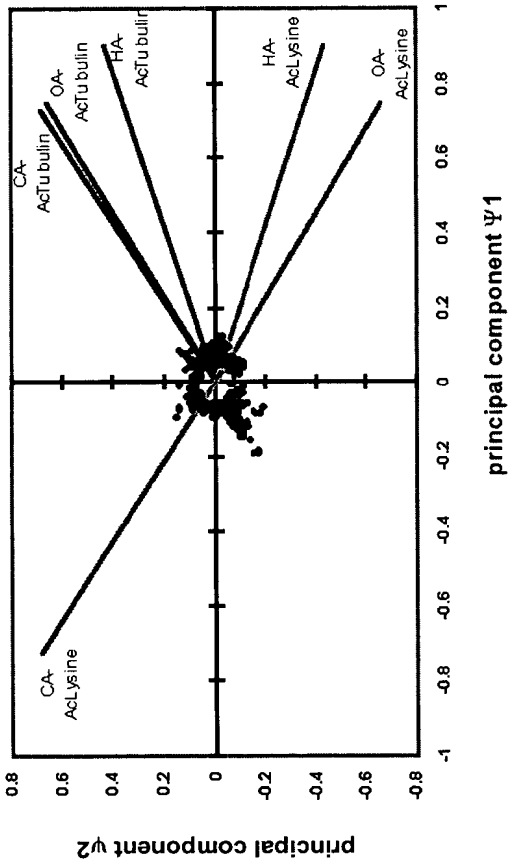


FIG. 34A

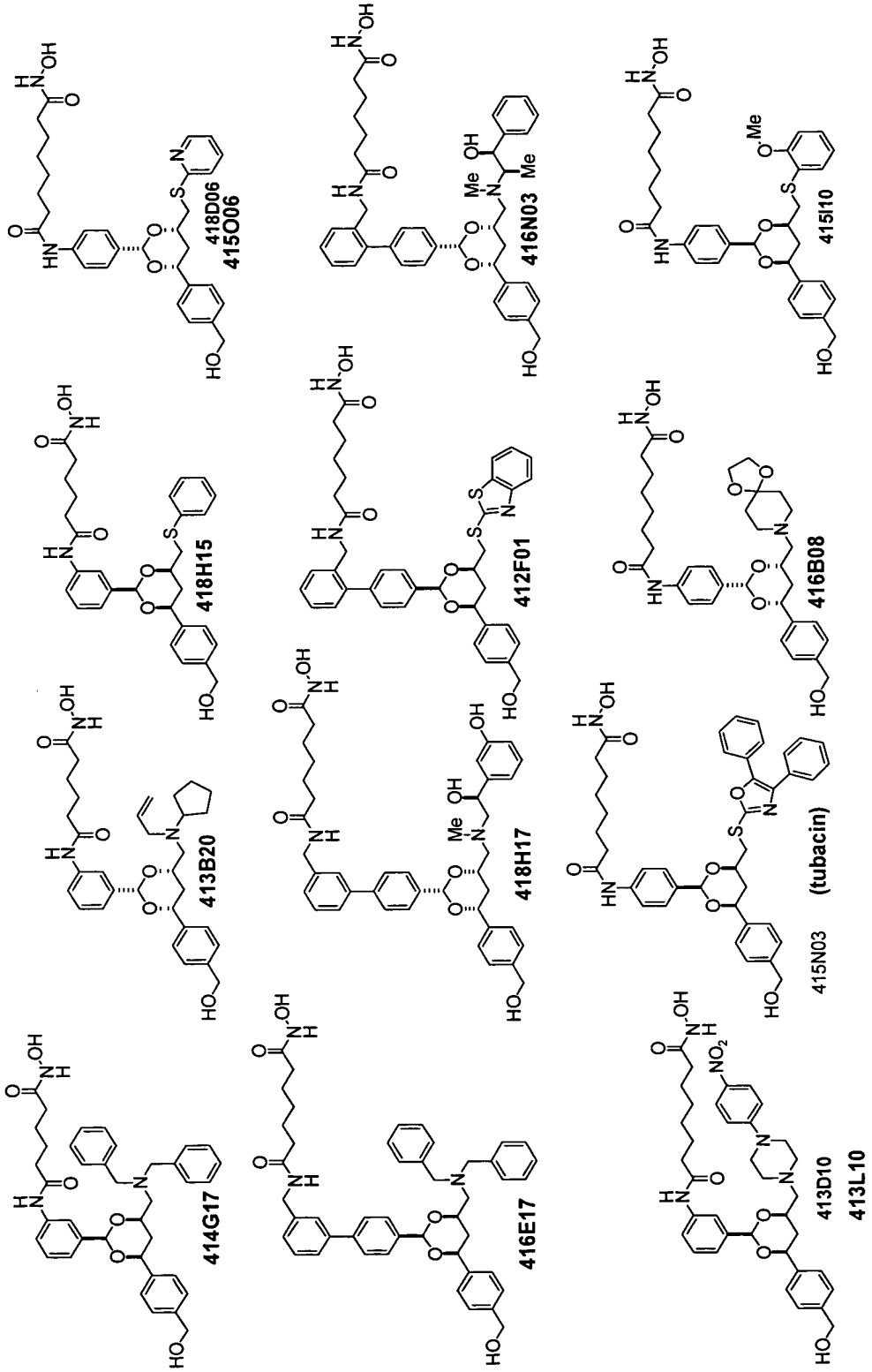

418D06
415O06
418H15
413B20
416N03
412F01
418H17
416B08
415I10
414G17
416E17
415N03 (tubacin)
413D10
413L10

FIG. 34B

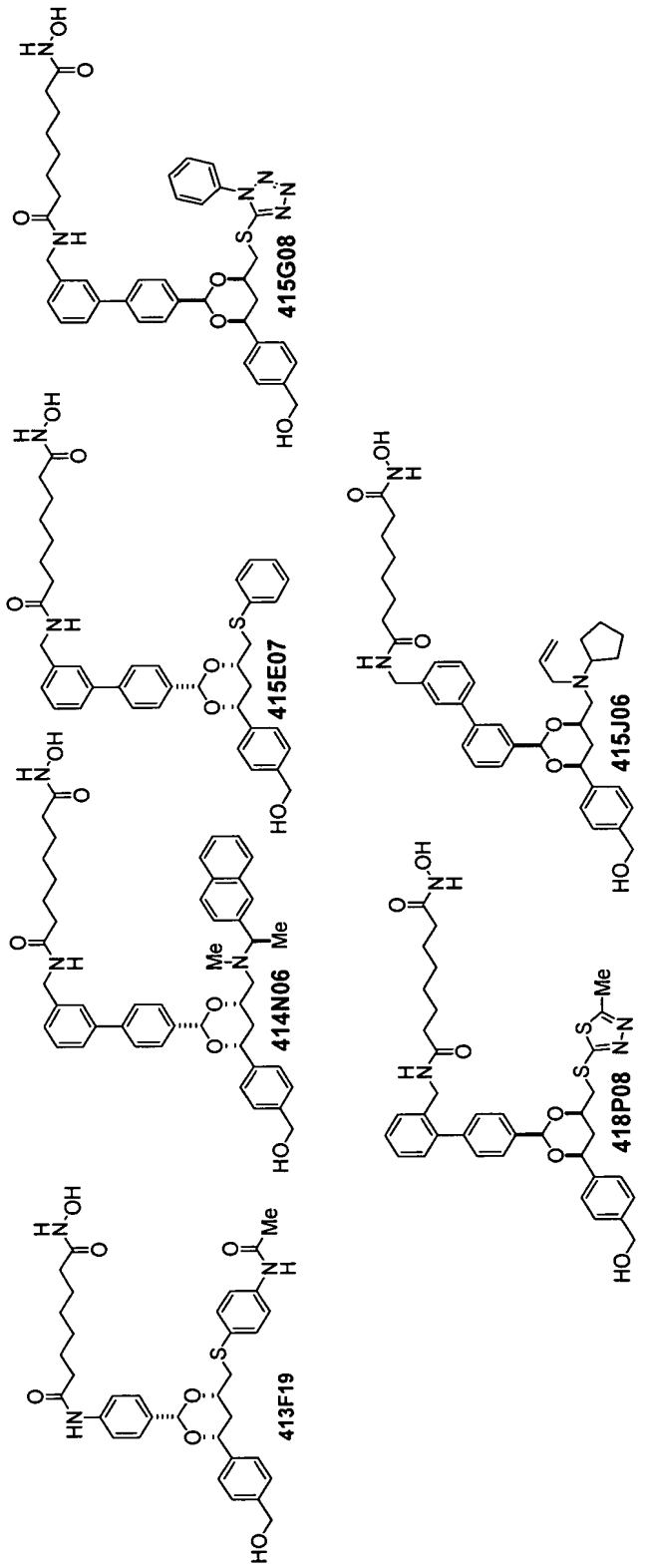


FIG. 35A

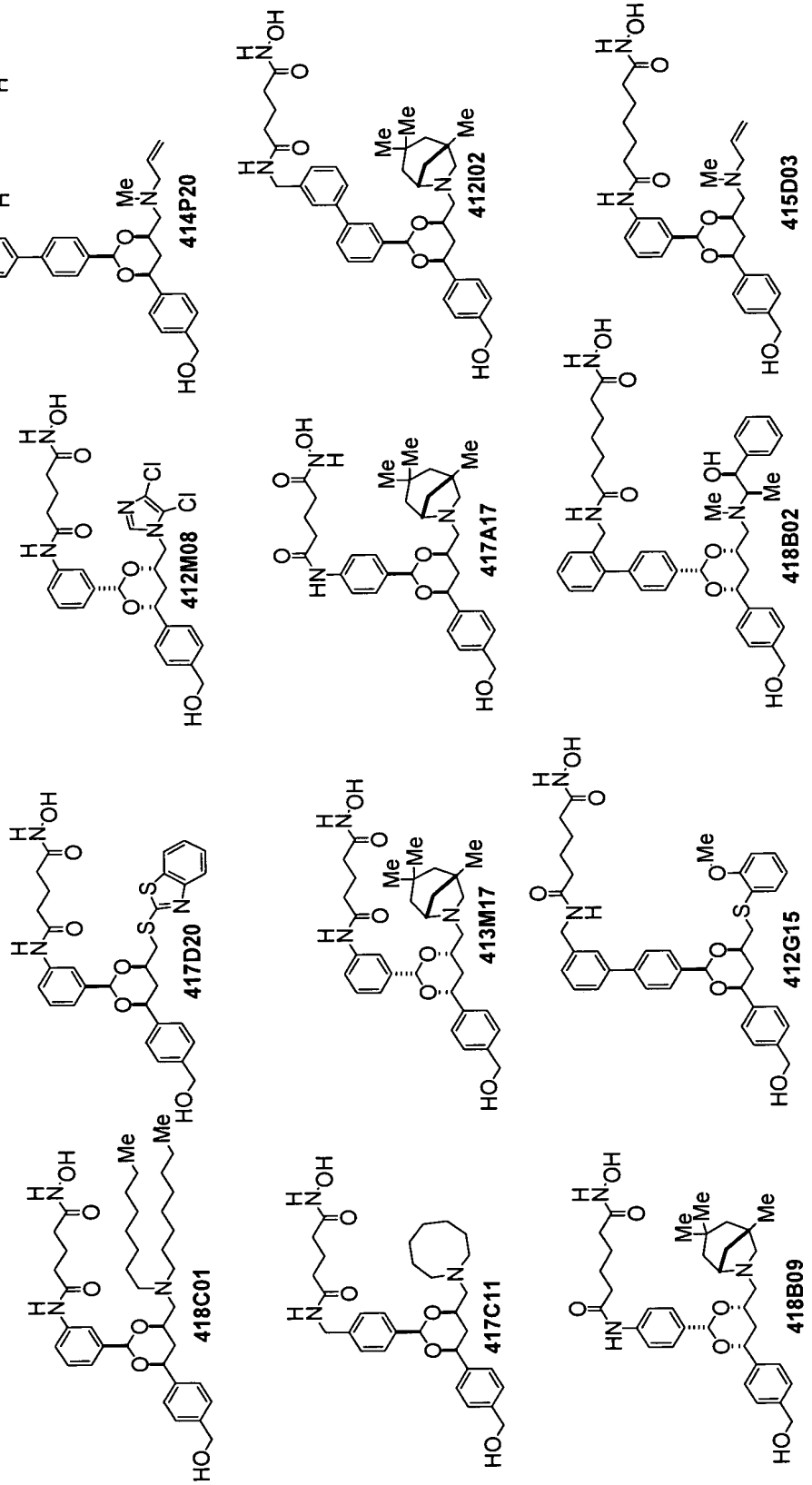

414P20
412M08
412I02
417A17
417D20
418B02
415D03
418C01
413M17
412G15
417C11
418B09

FIG. 35B

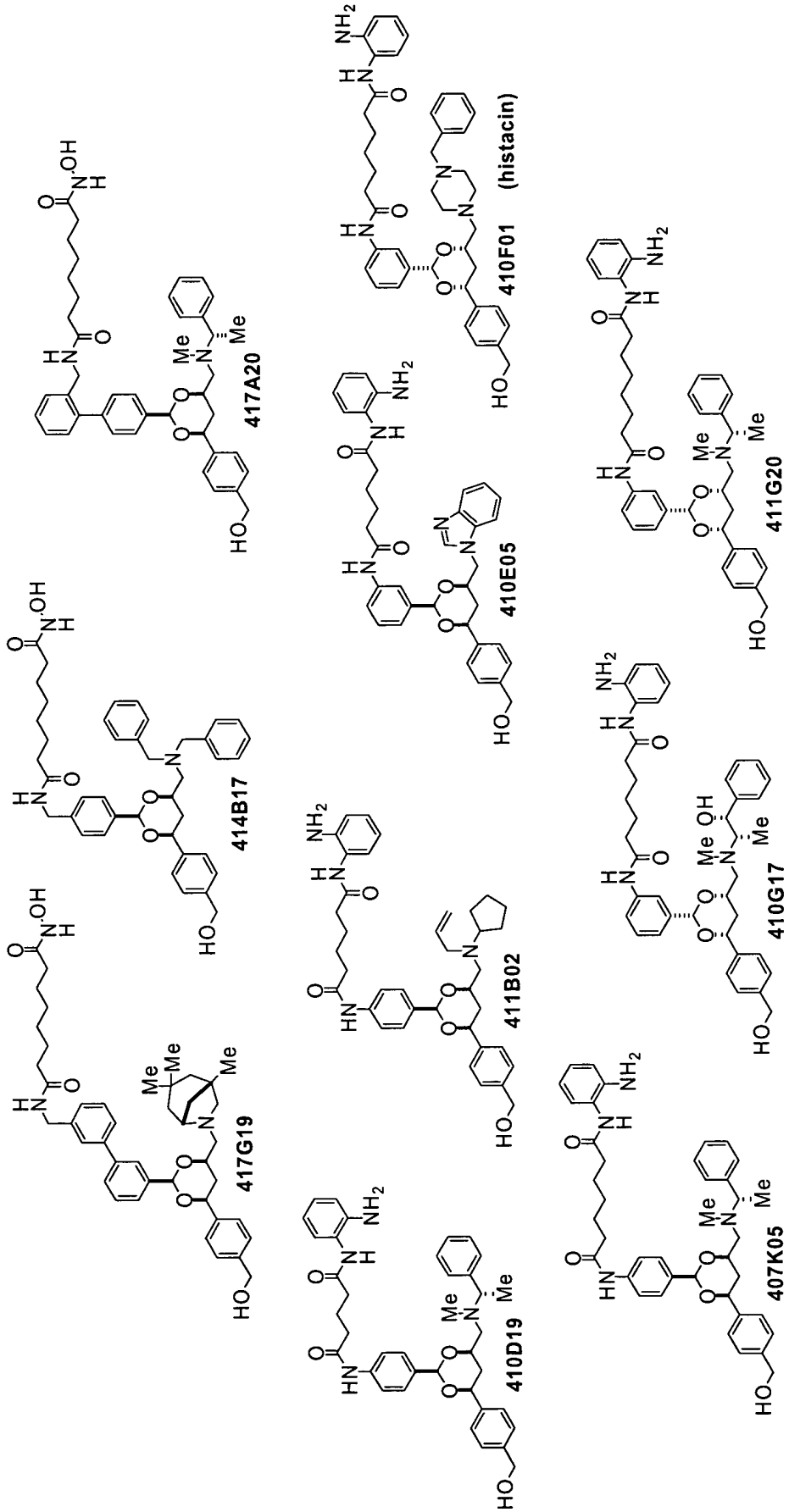
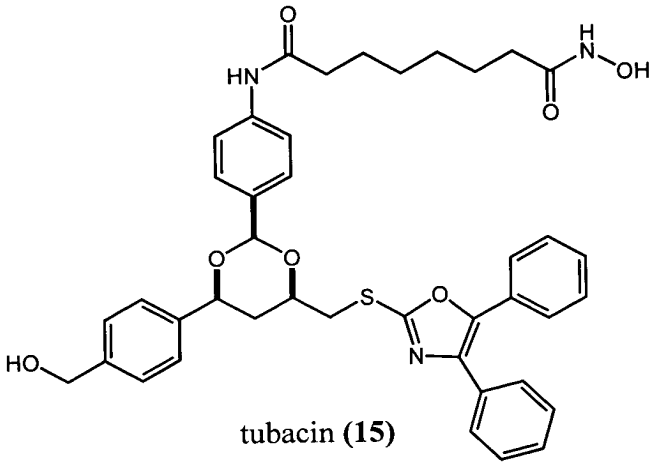

417A20
414B17
417G19
410F01 (histacin)
410E05
411B02
411G20
410G17
410D19
407K05

FIG. 36A



tubacin **(15)**

FIG. 36B

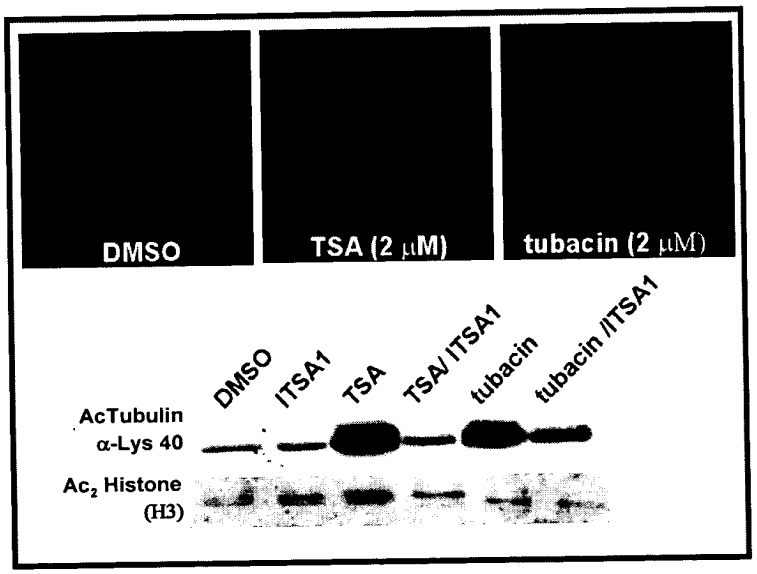
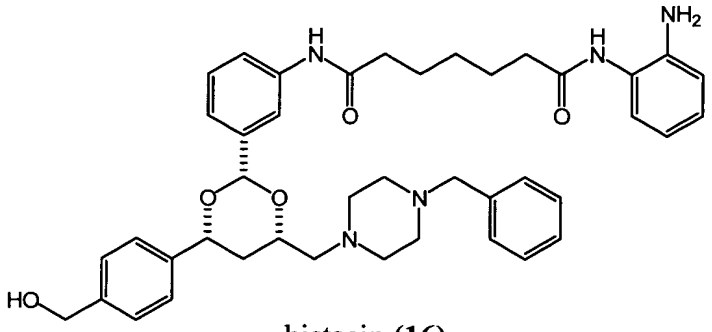


FIG. 36C



histacin **(16)**

FIG. 36D

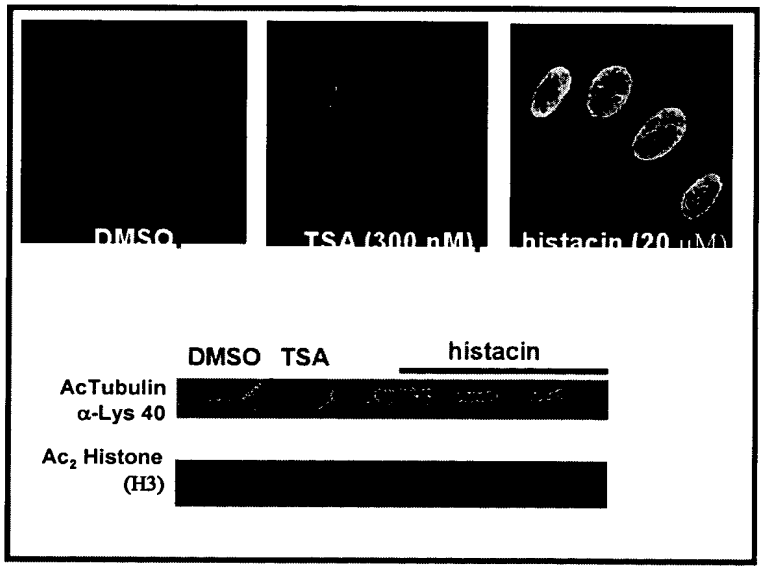


FIG. 36E

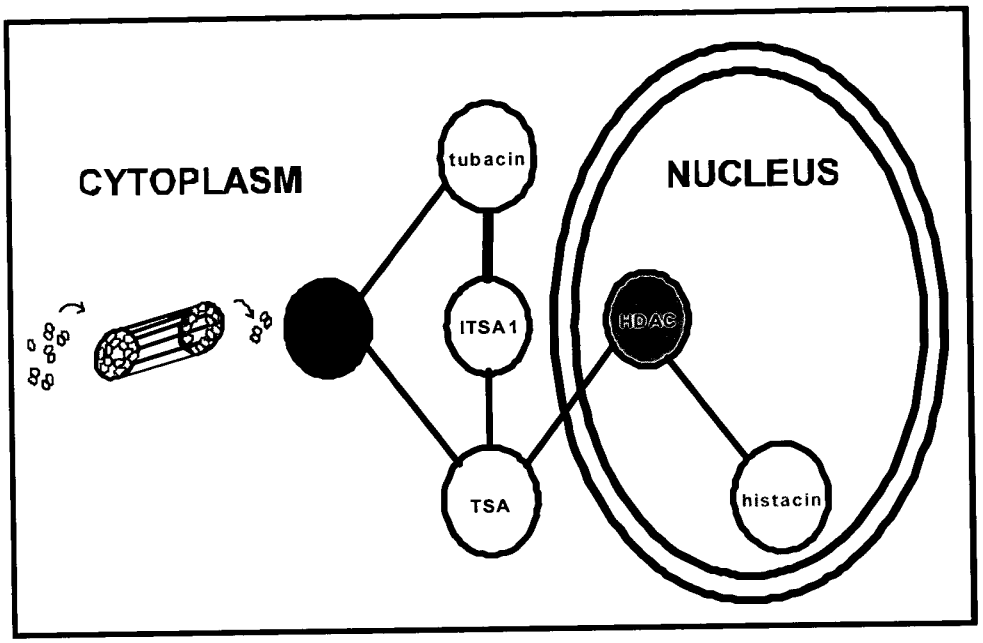


FIG. 37A

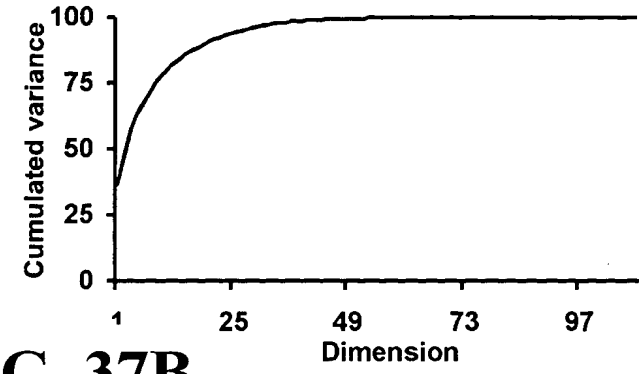


FIG. 37B

	F1	F2	F3	F4	F5
Eigenvalue	40.5	9.7	8.6	6.5	5.2
variance %	36.1	8.6	7.7	5.8	4.6
cumulated %	36.1	44.8	52.5	58.3	62.9

FIG. 37C

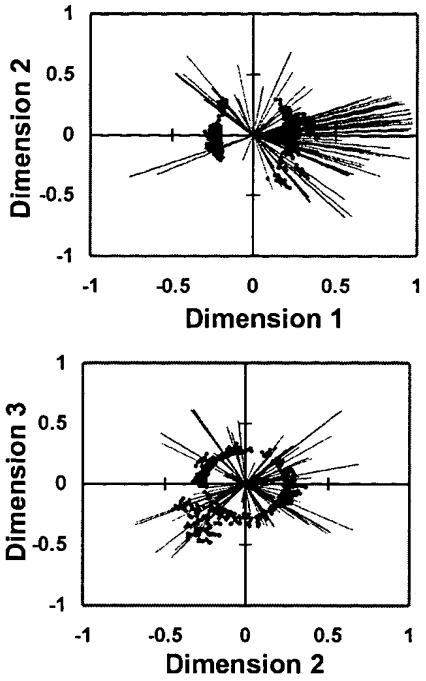


FIG. 37D

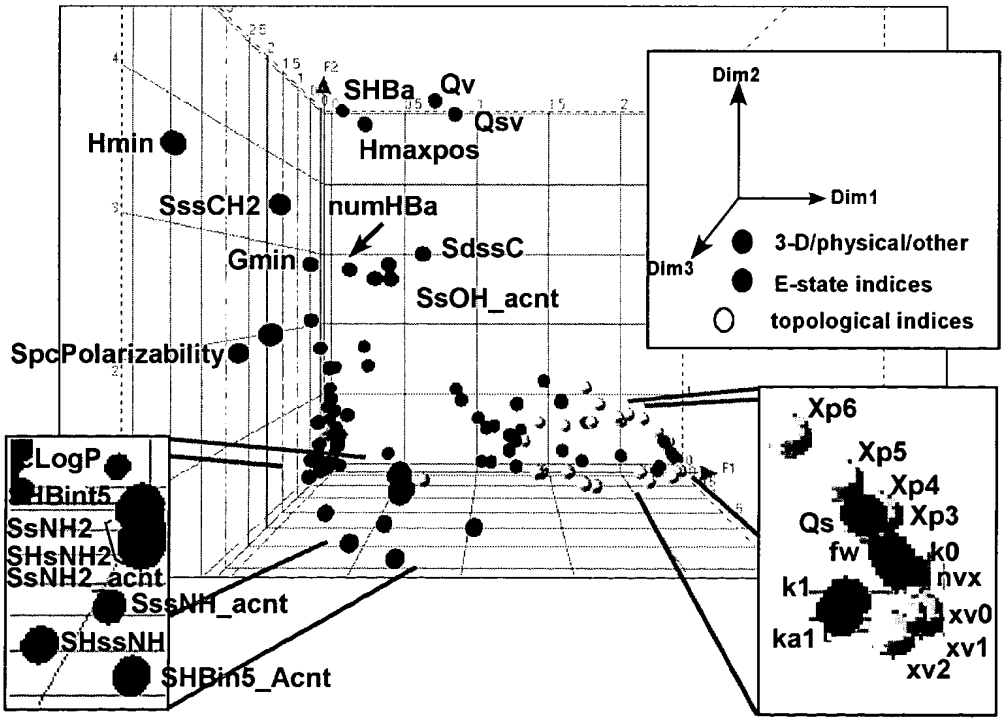


FIG. 38

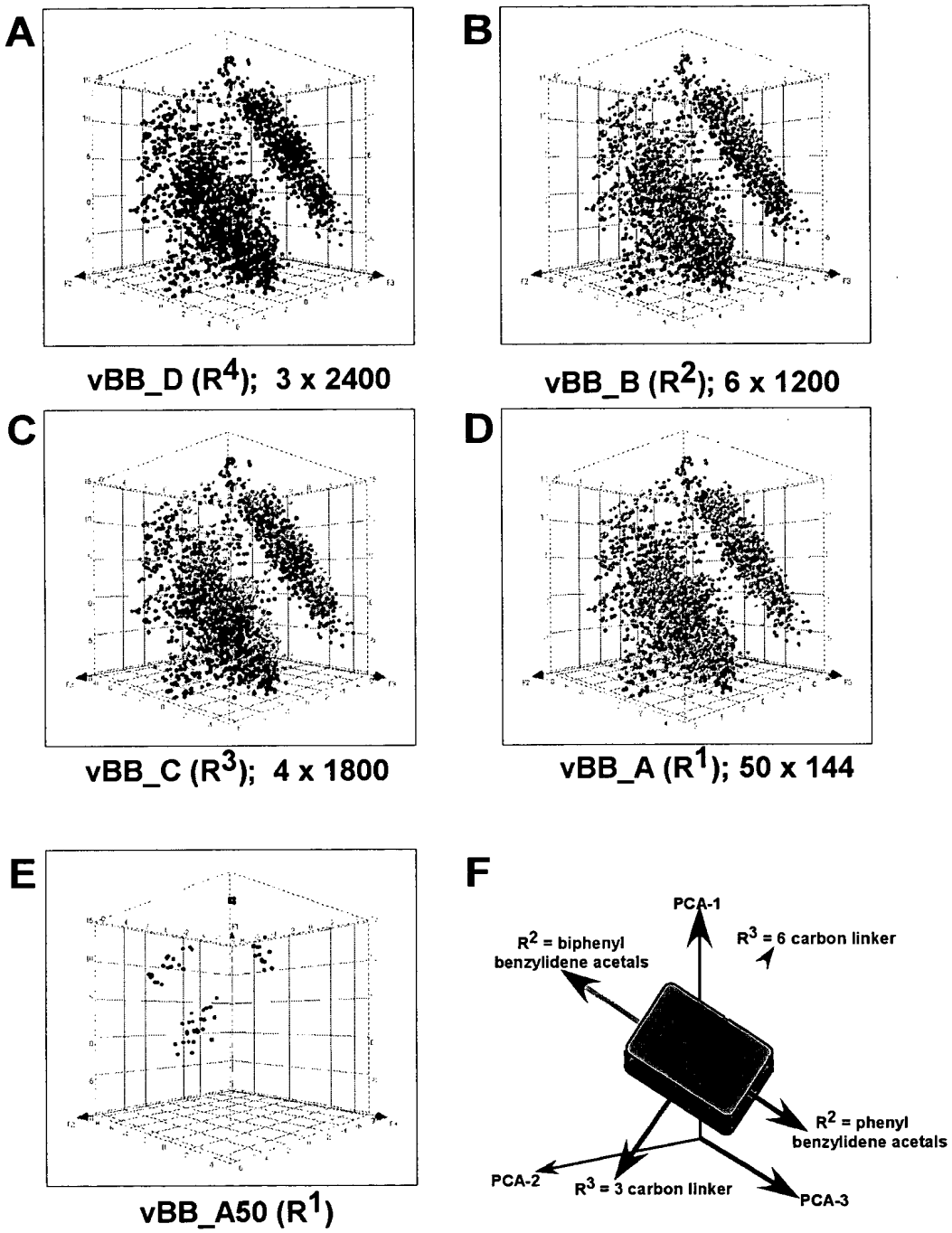

A
vBB_D (R^4); 3 x 2400
B
vBB_B (R^2); 6 x 1200
C
vBB_C (R^3); 4 x 1800
D
vBB_A (R^1); 50 x 144
E
vBB_A50 (R^1)
F
PCA-1
R^2 = biphenyl benzylidene acetals
R^3 = 6 carbon linker
R^2 = phenyl benzylidene acetals
PCA-2
R^3 = 3 carbon linker
PCA-3

FIG. 39

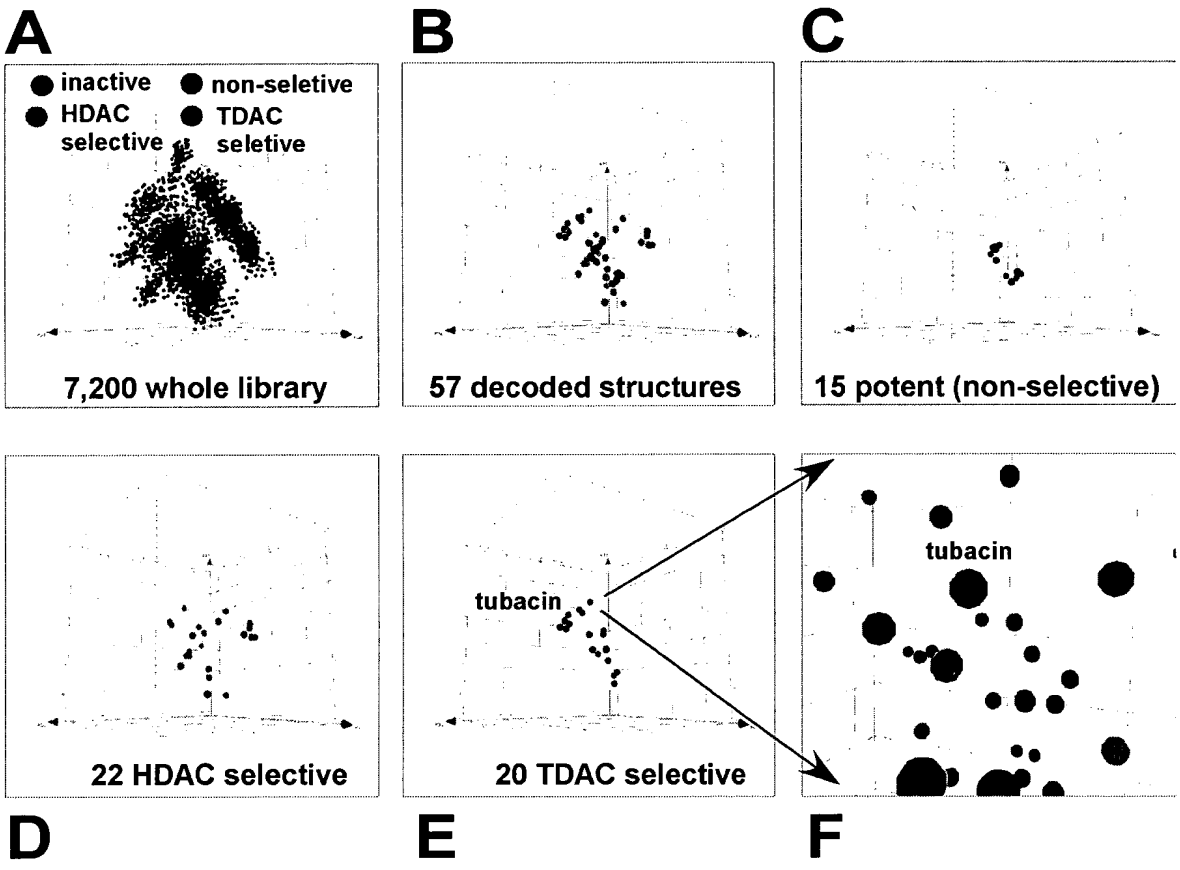

A
inactive
non-seletive
HDAC selective
TDAC seletive
7,200 whole library
B
57 decoded structures
C
15 potent (non-selective)
22 HDAC selective
tubacin
20 TDAC selective
tubacin
D
E
F

FIG. 40

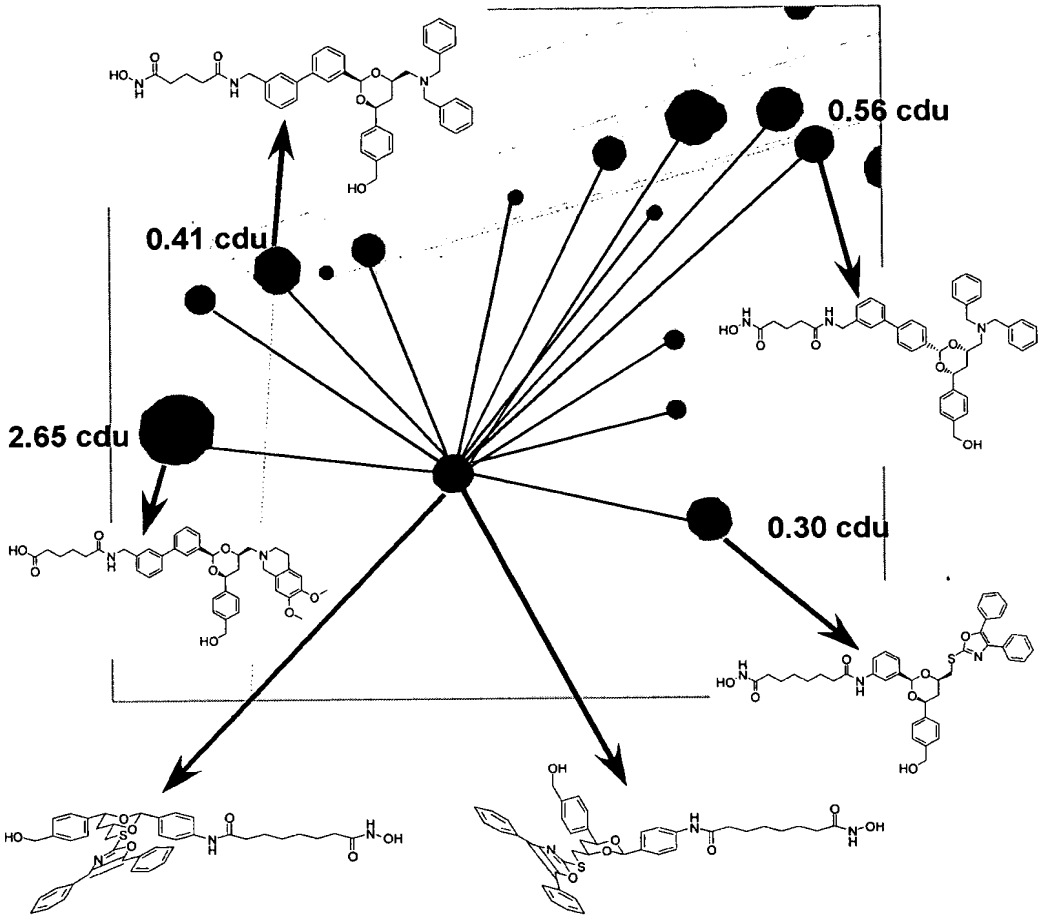


FIG. 41A

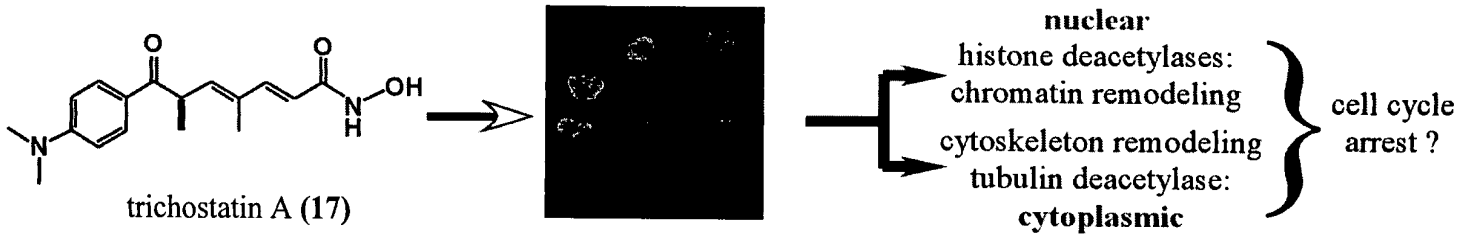


FIG. 41B

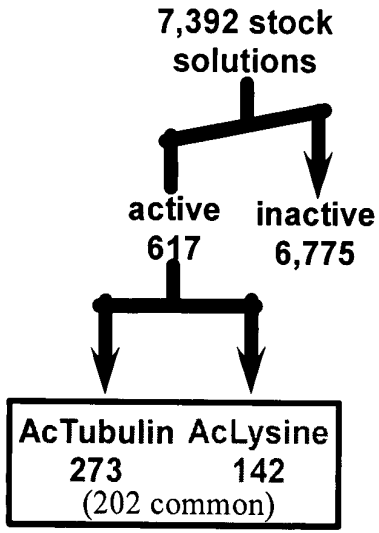


FIG. 41C

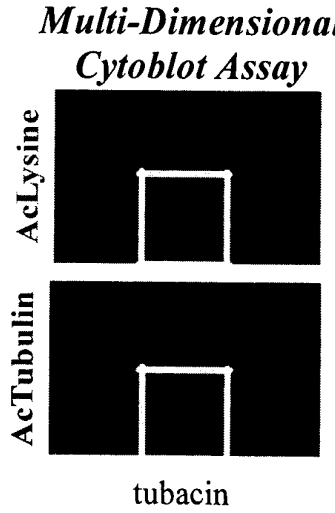
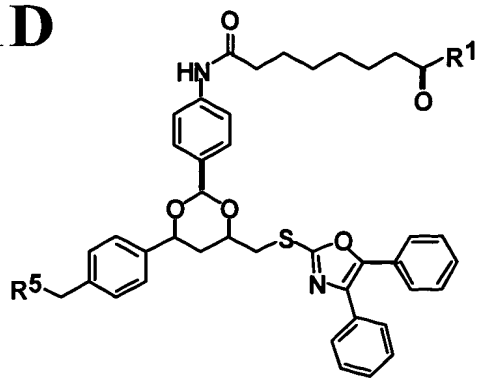


FIG. 41D



R^1 = 	R^1 = -OH	R^1 = 
R^5 = -OH	R^5 = -OH	R^5 = -PEG-biotin
tubacin **(15)**	niltubacin **(18)**	biotubacin **(19)**

FIG. 41E

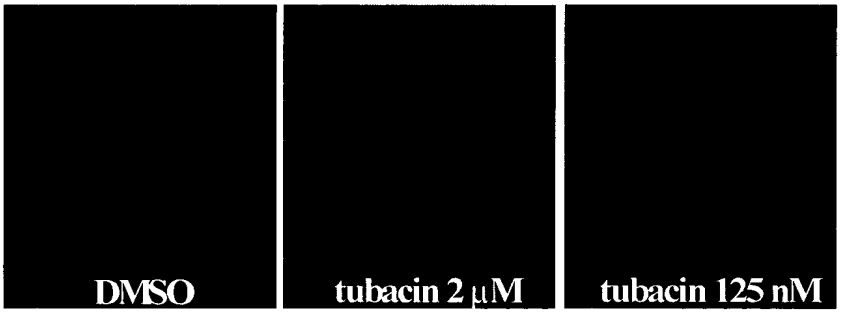


FIG. 41F

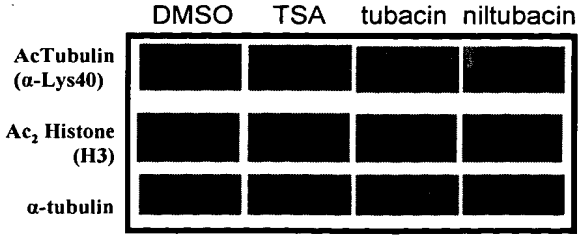


FIG. 42A

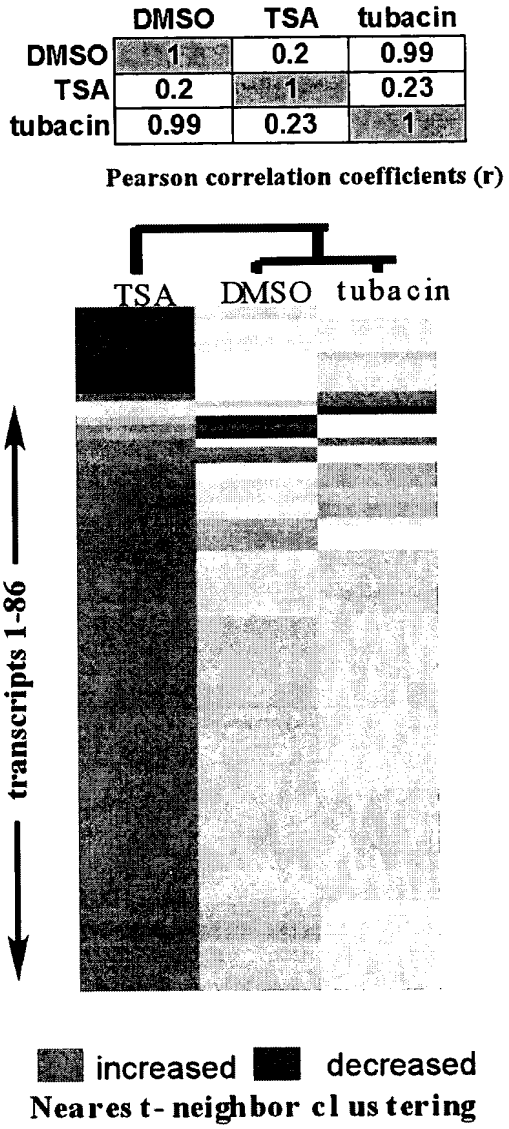

DMSO
TSA
tubacin
DMSO 1 0.2 0.99
TSA 0.2 1 0.23
tubacin 0.99 0.23 1
Pearson correlation coefficients (r)
TSA
DMSO
tubacin
transcripts 1-86
increased
decreased
Nearest-neighbor clustering

FIG. 42B

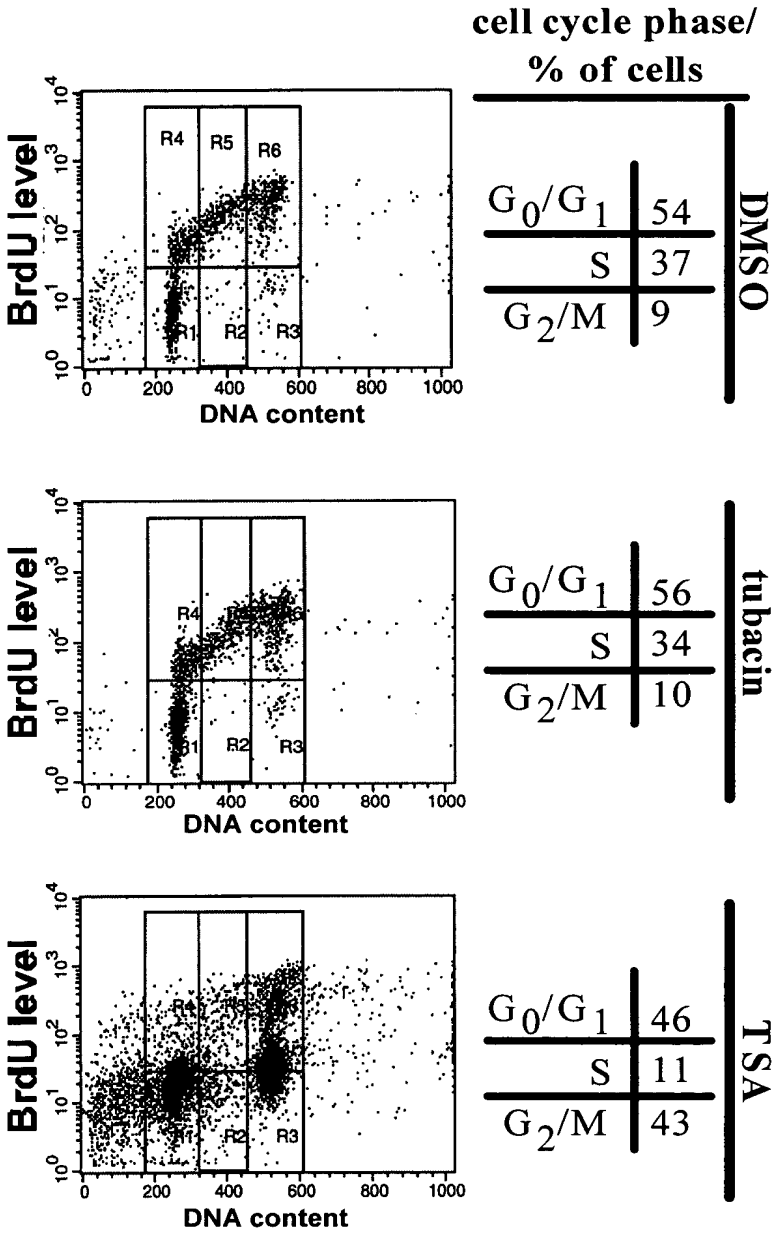

cell cycle phase/
% of cells
BrdU level
DNA content
R1
R2
R3
R4
R5
R6
G0/G1
S
G2/M
54
37
9
DMSO
56
34
10
tubacin
46
11
43
TSA

FIG. 42C

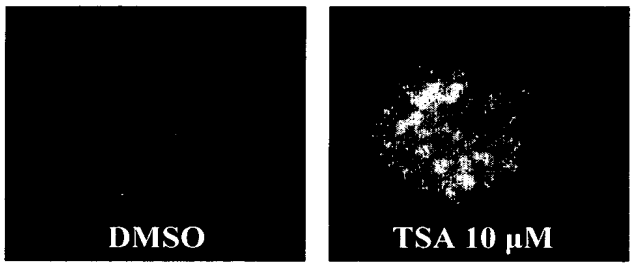


FIG. 42D

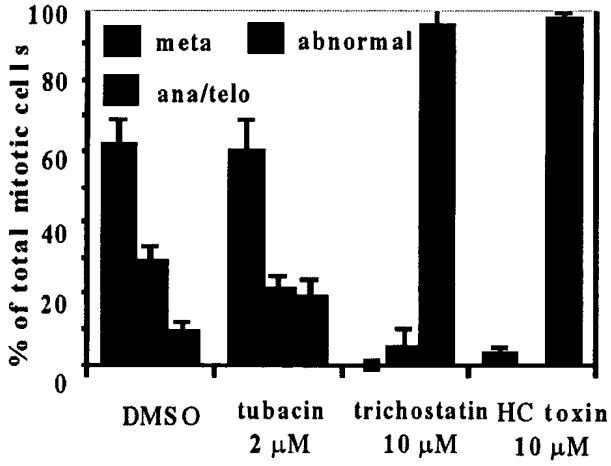


FIG. 43A

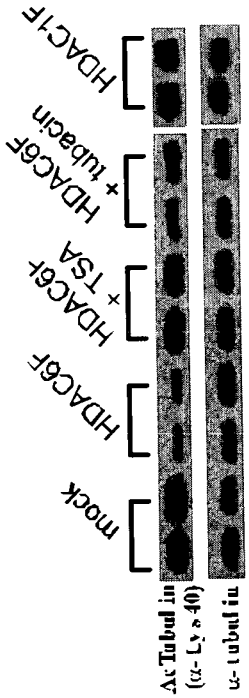


FIG. 43B

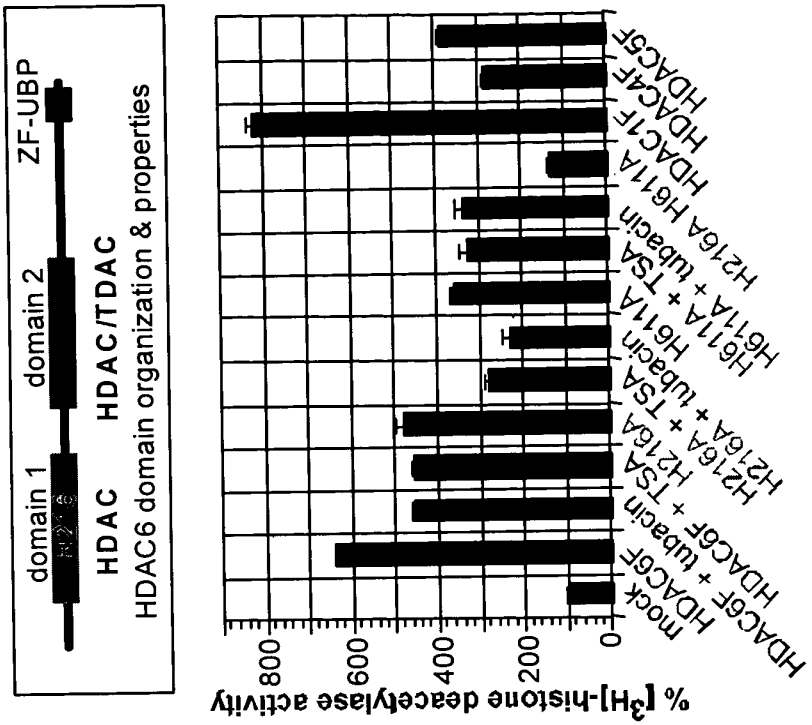


FIG. 43C

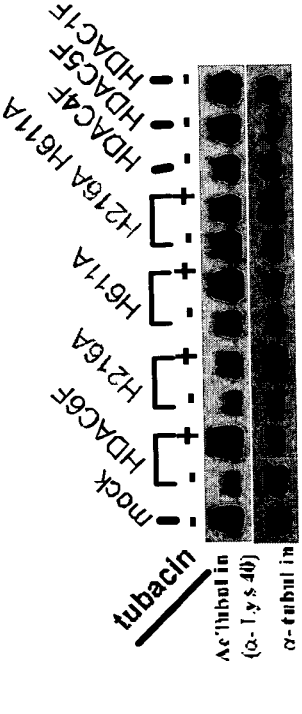


FIG. 43D

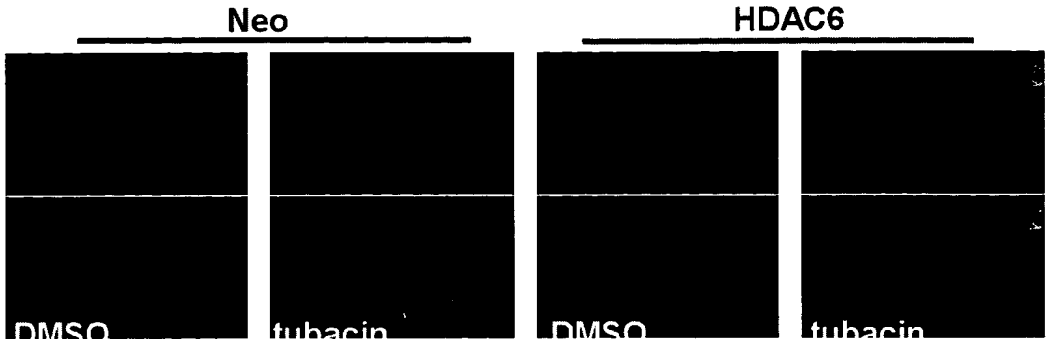


FIG. 43E

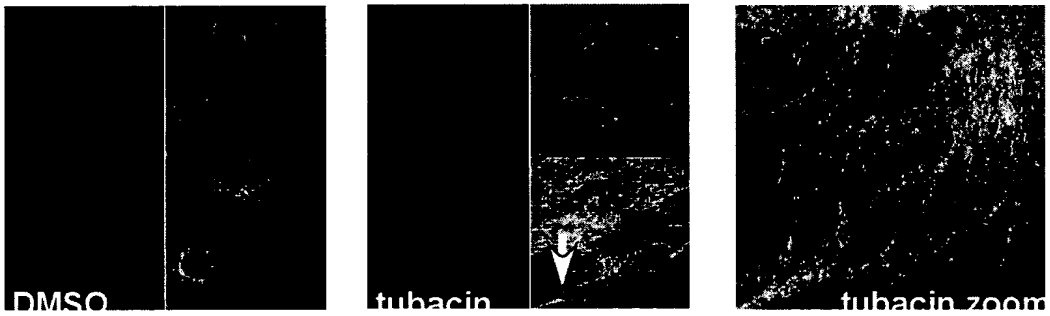


FIG. 43F

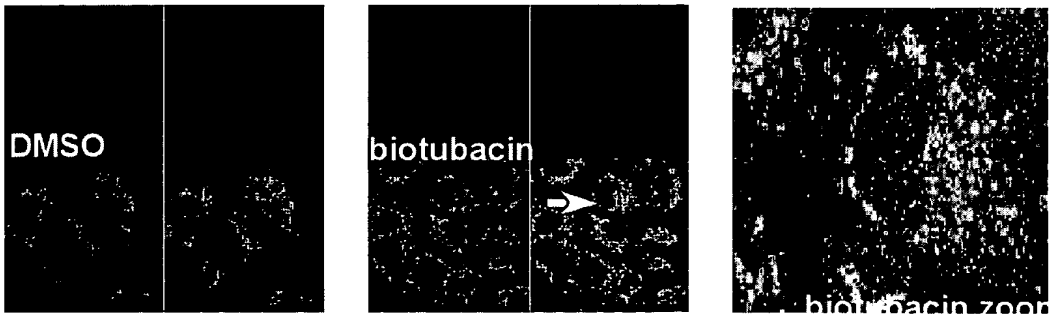
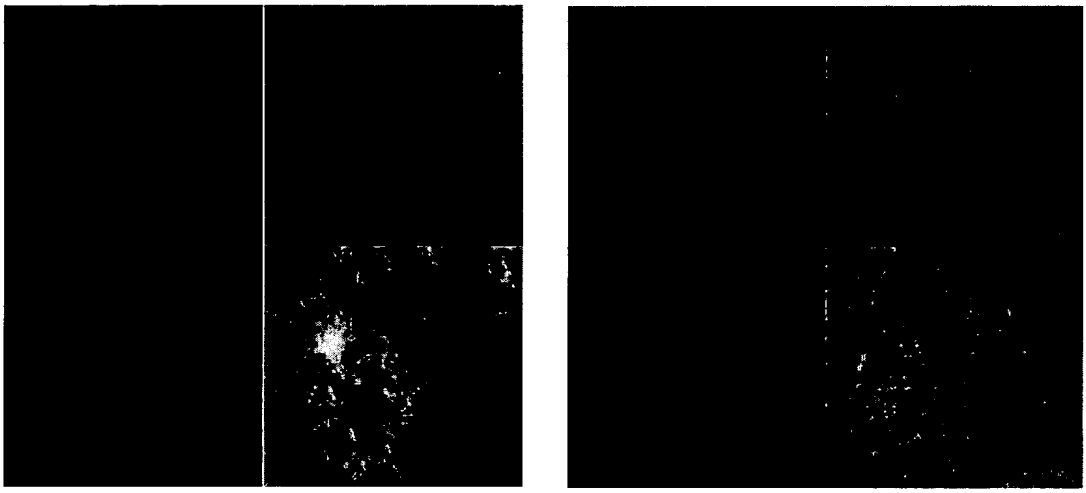


FIG. 44A



DMSO pre-treatment

tubacin (2 μM) pre-treatment

FIG. 44B

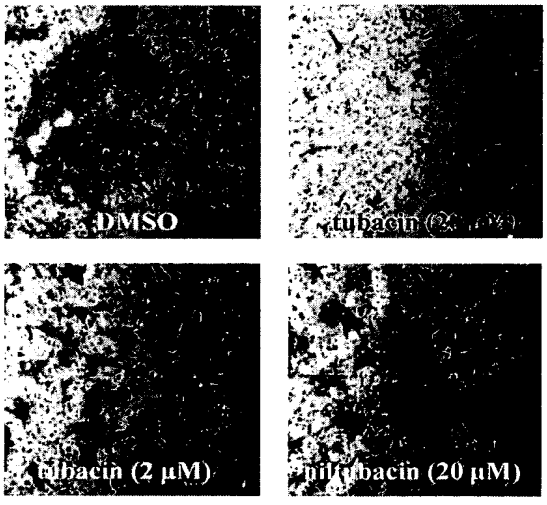


FIG. 44C

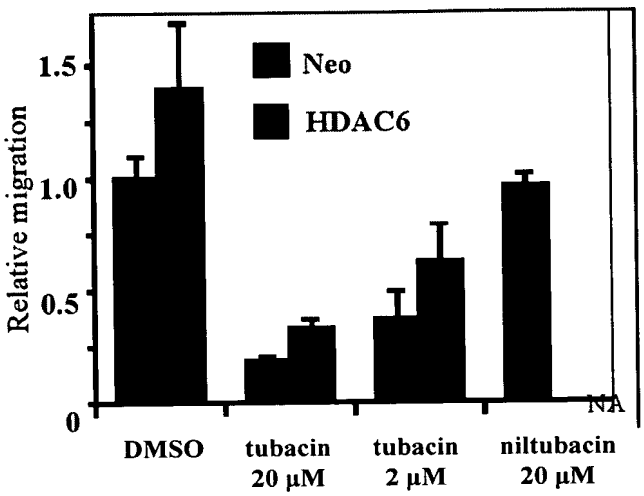


FIG. 44D

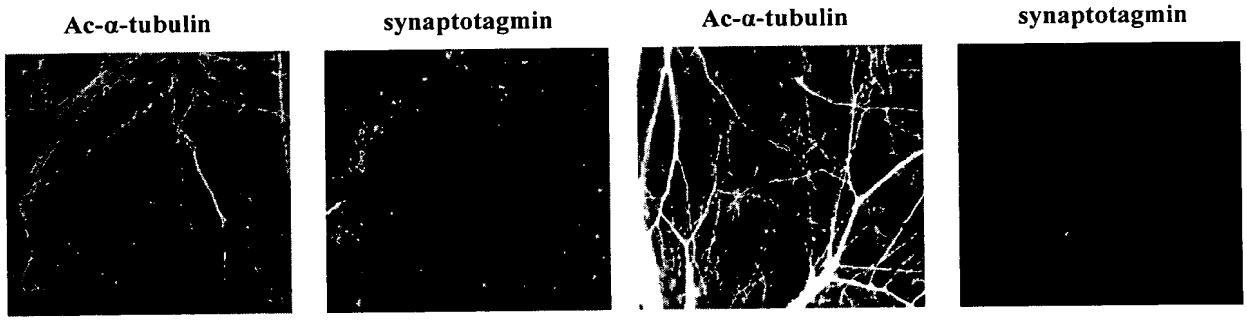


FIG. 45A

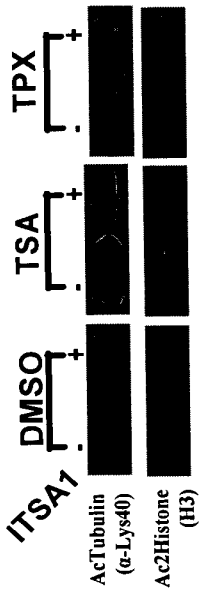
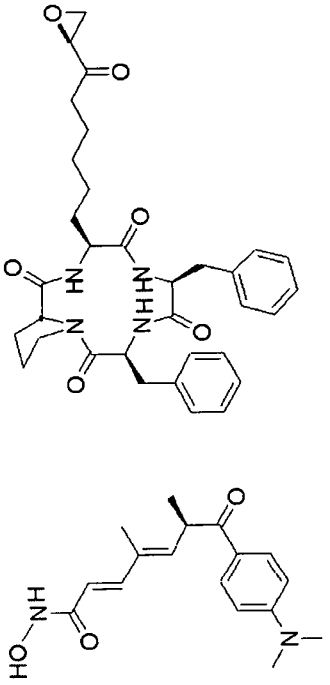


FIG. 45B



(20)

(17)

FIG. 45C

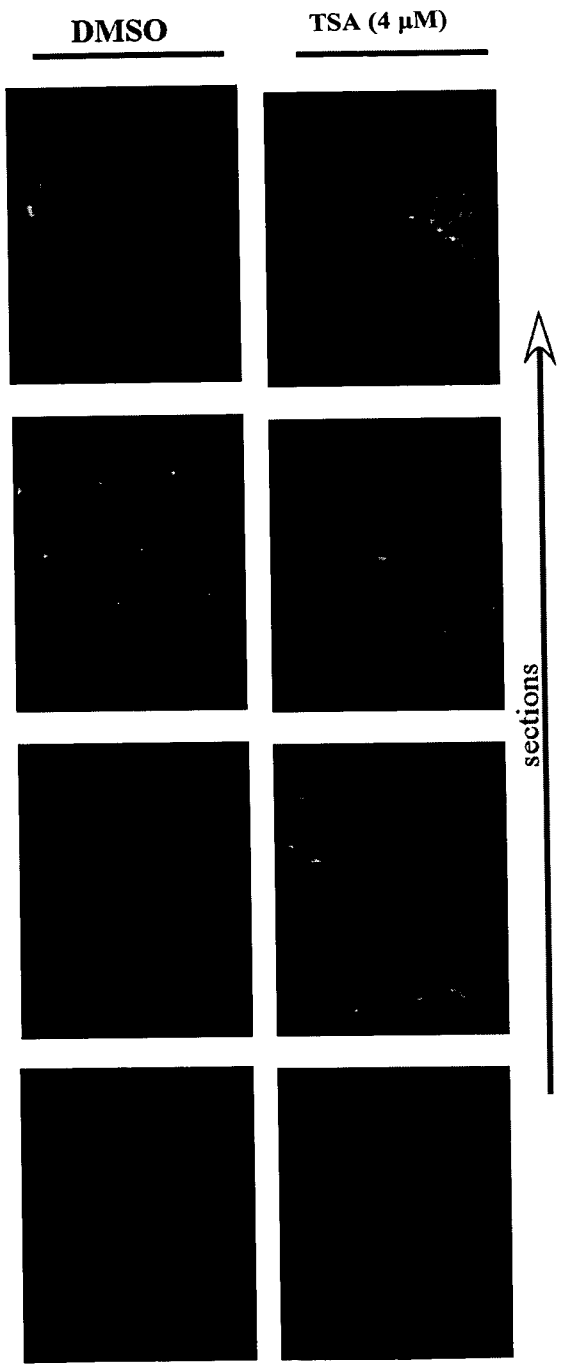
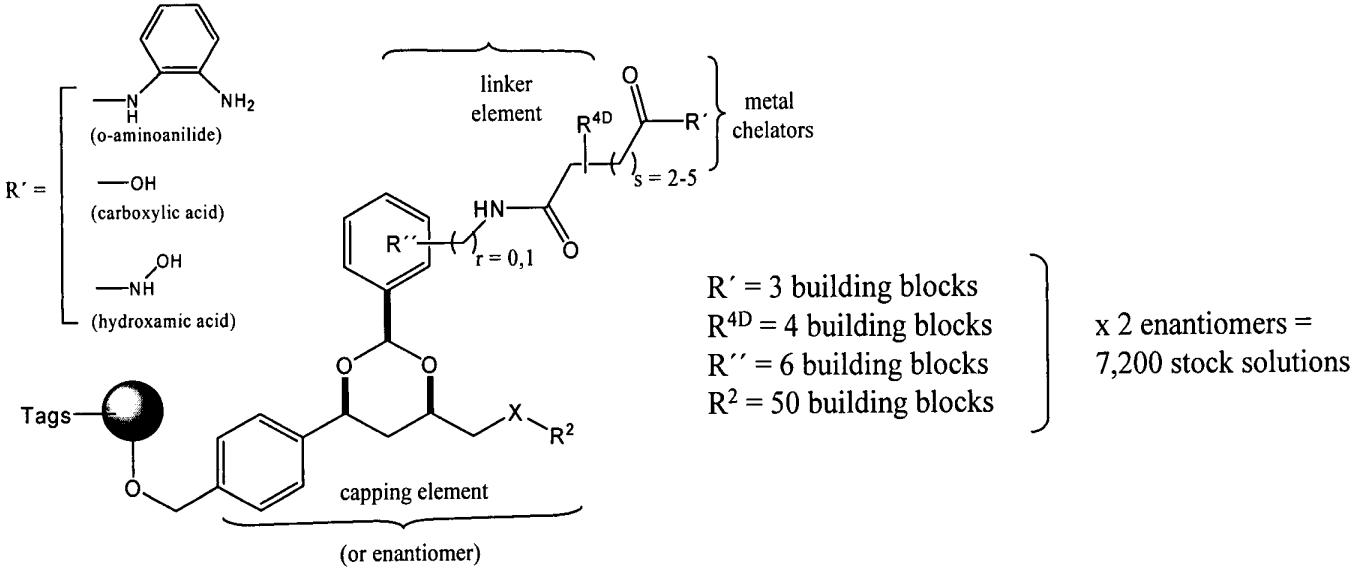


FIG. 45D



Biasing elements in diversity-oriented synthesis

FIG. 46

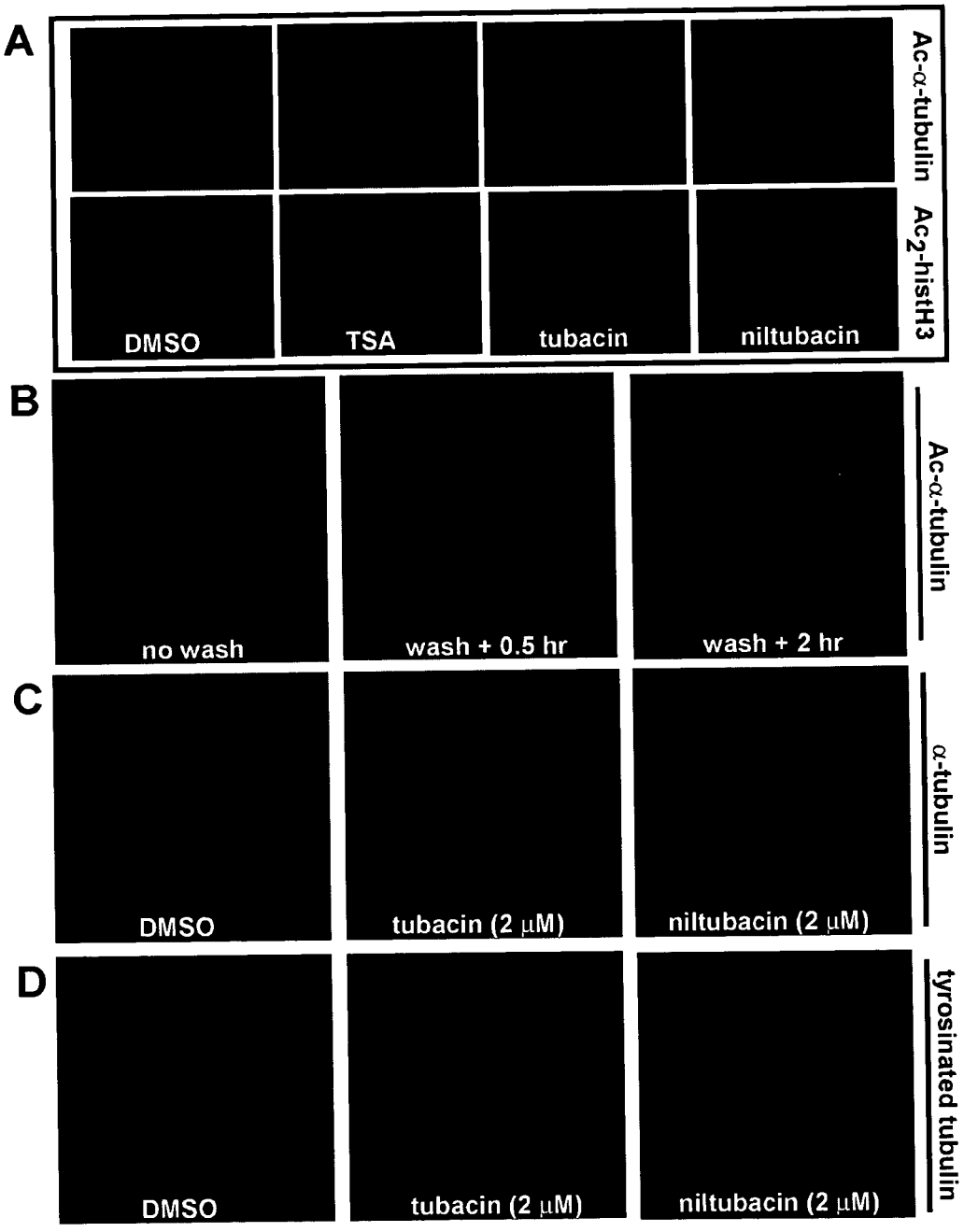
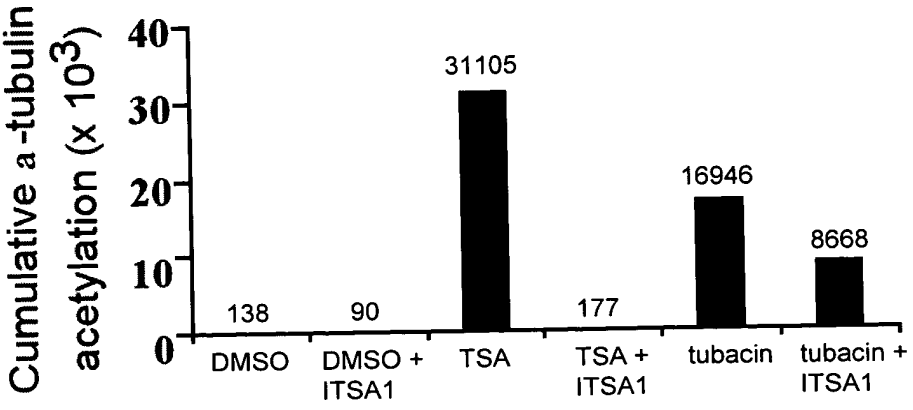

A
Ac-α-tubulin
Ac2-histH3
DMSO
TSA
tubacin
niltubacin
B
Ac-α-tubulin
no wash
wash + 0.5 hr
wash + 2 hr
C
α-tubulin
DMSO
tubacin (2 μM)
niltubacin (2 μM)
D
tyrosinated tubulin
DMSO
tubacin (2 μM)
niltubacin (2 μM)

FIG. 47A

Treatment	Mean α-tubulin acetylation
DMSO	92
ITSA1	82
TSA	322
TSA + ITSA1	104
tubacin	183
tubacin + ITSA1	110

FIG. 47B



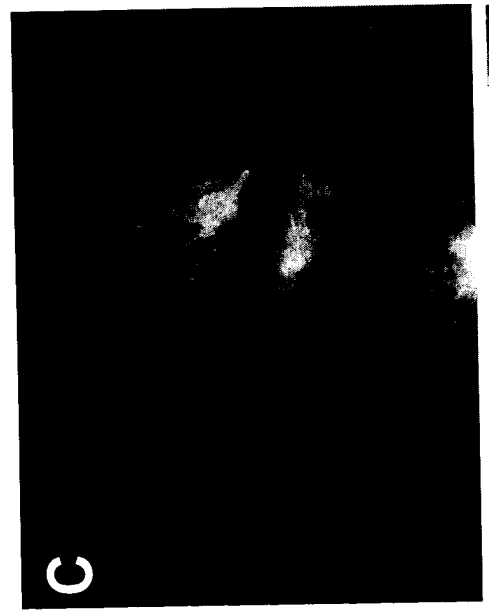


FIG. 47

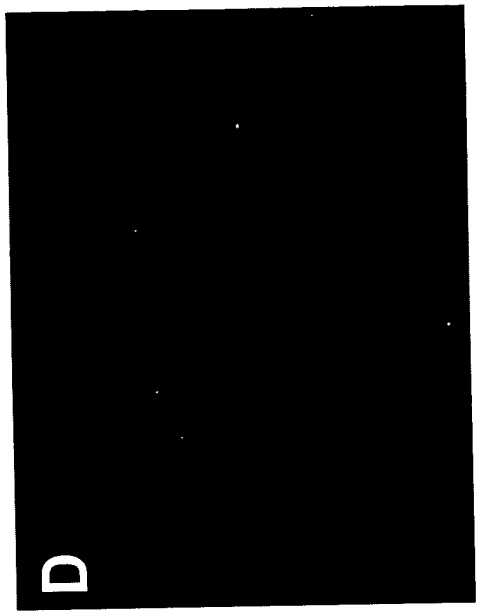


FIG. 47

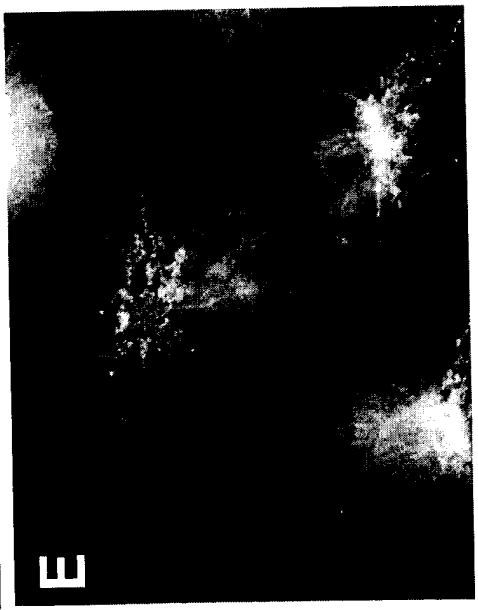


FIG. 47

FIG. 47G

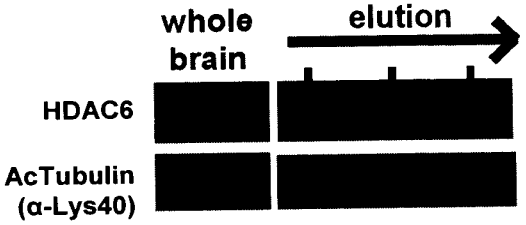


FIG. 47H

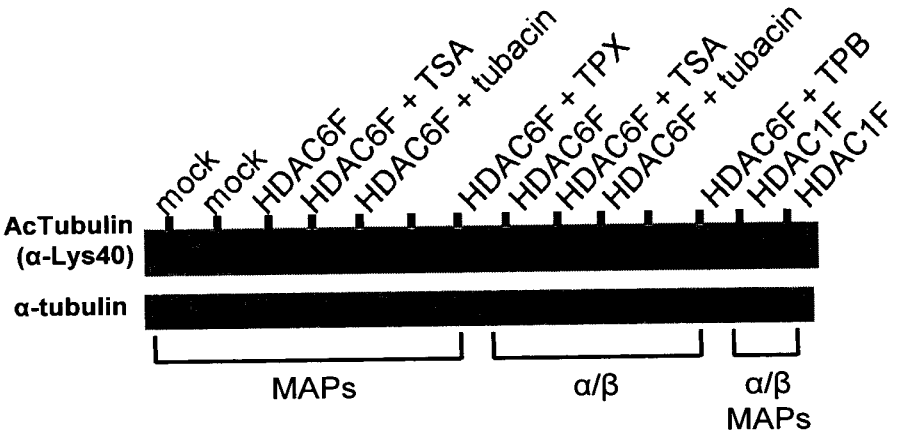


FIG. 48A

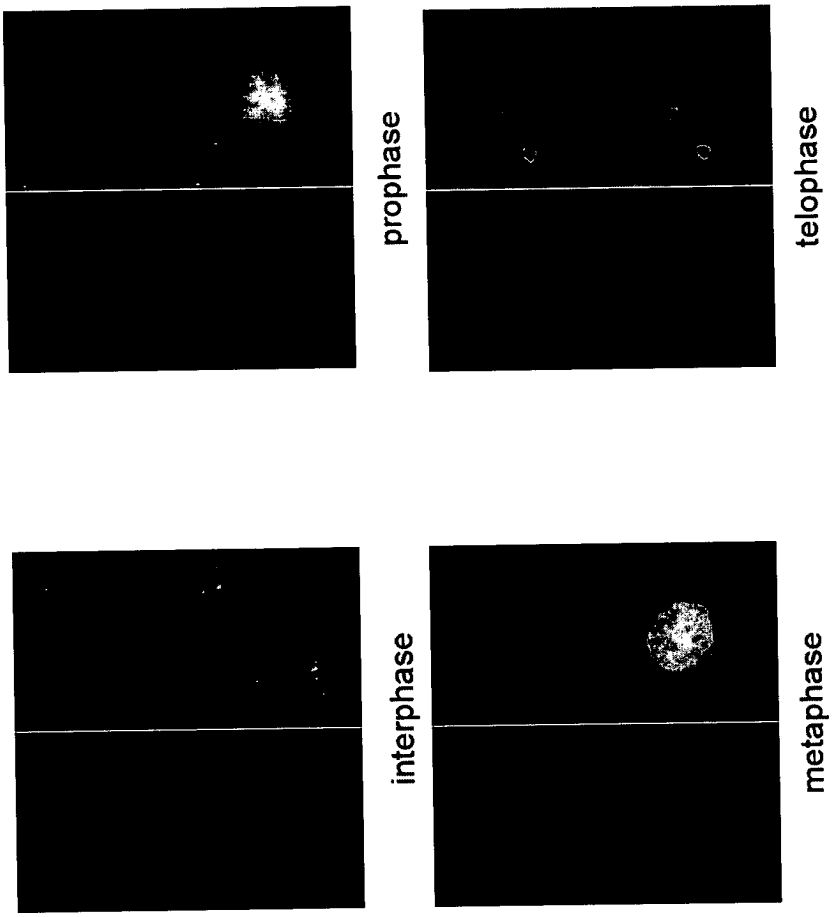


FIG. 48B

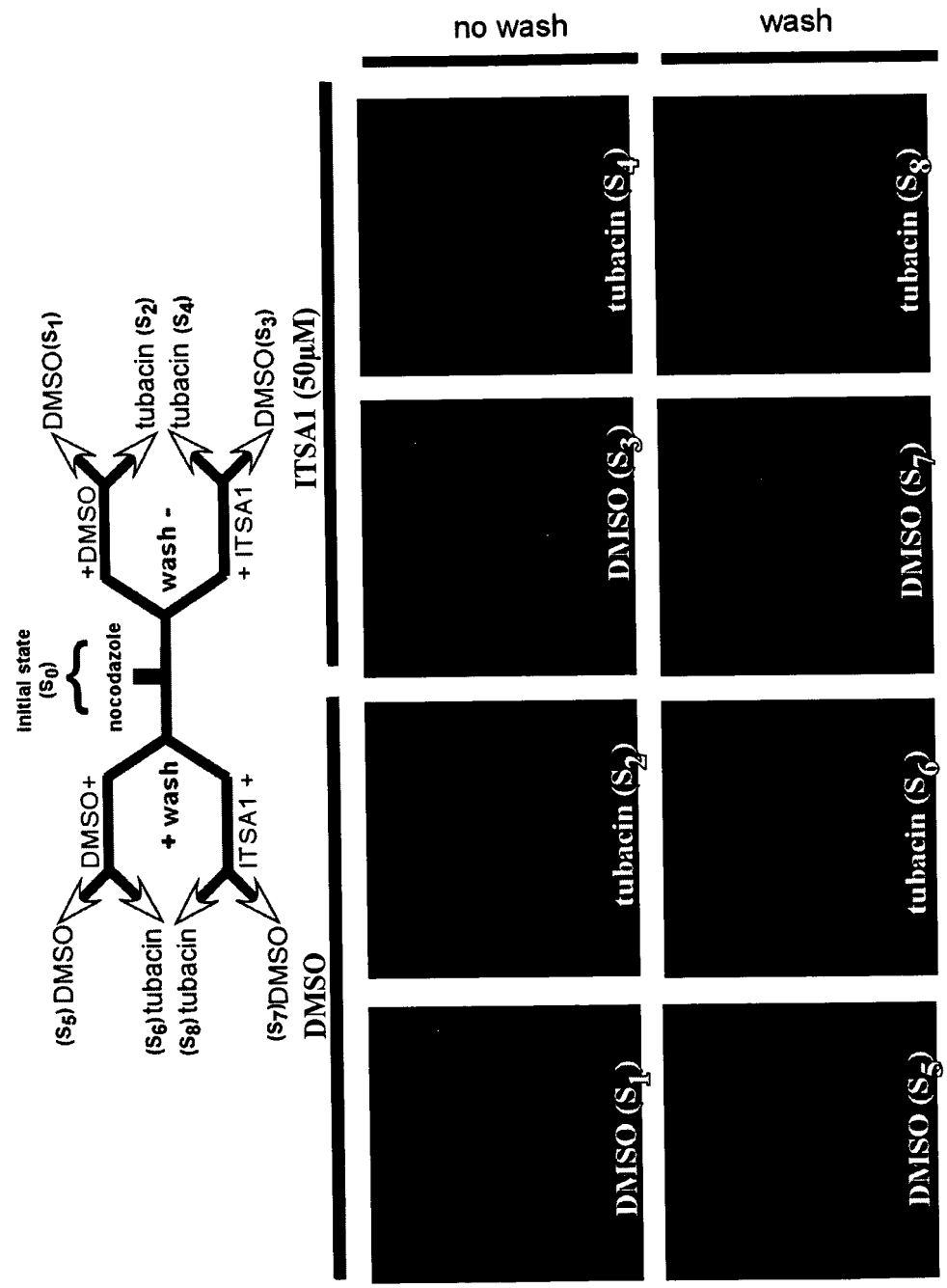

Initial state (S_0)
nocodazole
wash -
+DMSO
+ ITSA1
DMSO(S_1)
tubacin (S_2)
tubacin (S_4)
DMSO(S_3)
ITSA1 (50μM)
+ wash
DMSO+
ITSA1 +
(S_5) DMSO
(S_6) tubacin
(S_8) tubacin
(S_7)DMSO
DMSO
no wash
wash
tubacin (S_4)
DMSO (S_3)
tubacin (S_2)
DMSO (S_1)
tubacin (S_8)
DMSO (S_7)
tubacin (S_6)
DMSO (S_5)

FIG. 49A

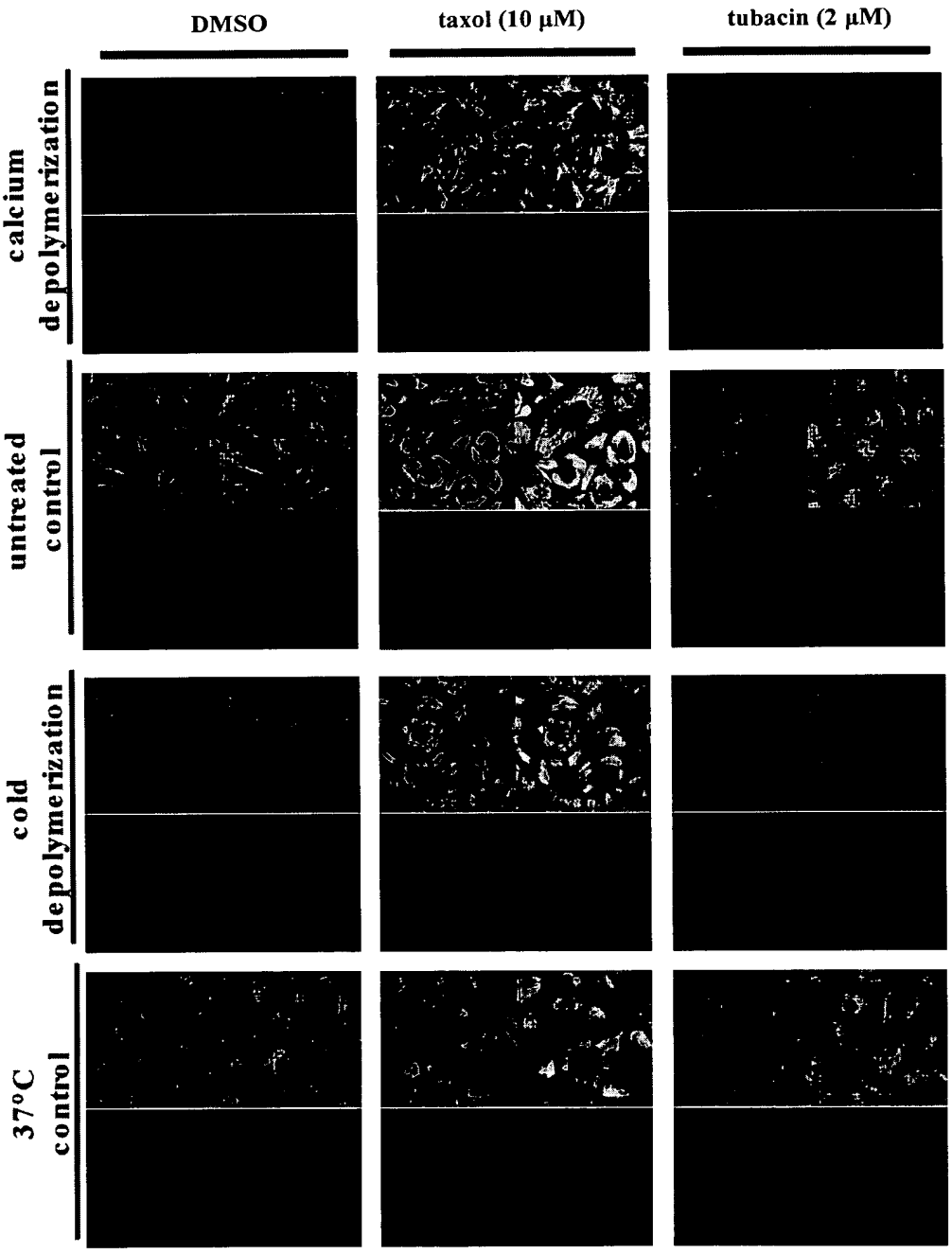

DMSO
taxol (10 μM)
tubacin (2 μM)
calcium depolymerization
untreated control
cold depolymerization
37°C control

FIG. 49B

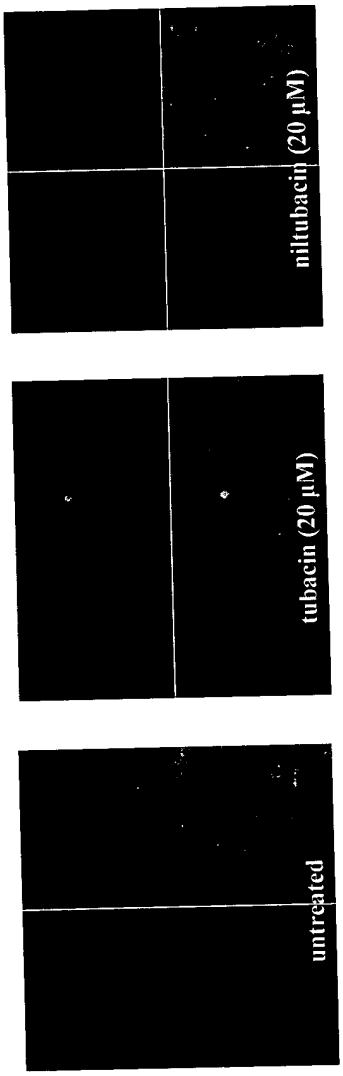


FIG. 49C

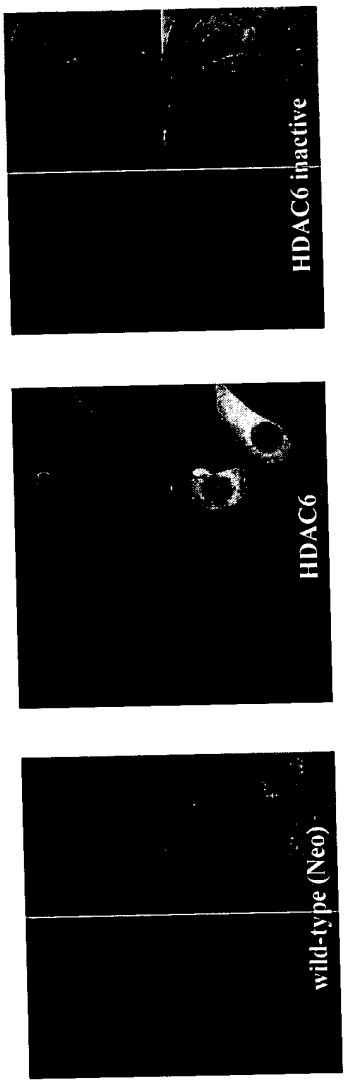


FIG. 50A

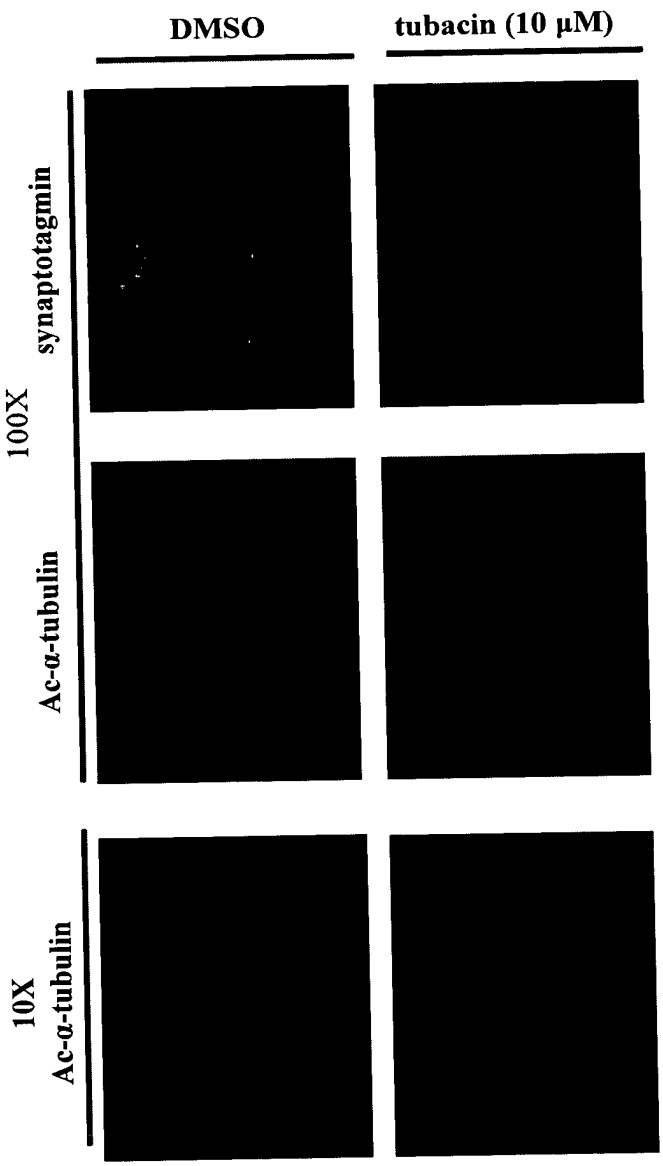

DMSO
tubacin (10 μM)
100X
synaptotagmin
Ac-α-tubulin
10X
Ac-α-tubulin

FIG. 50B

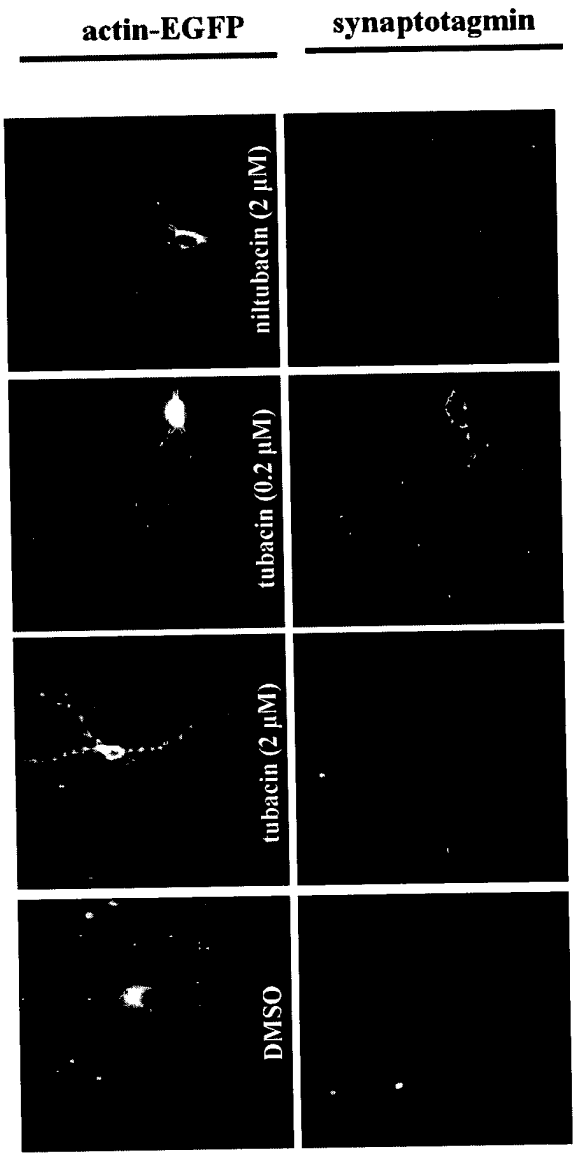

actin-EGFP
synaptotagmin
niltubacin (2 μM)
tubacin (0.2 μM)
tubacin (2 μM)
DMSO

FIG. 50C

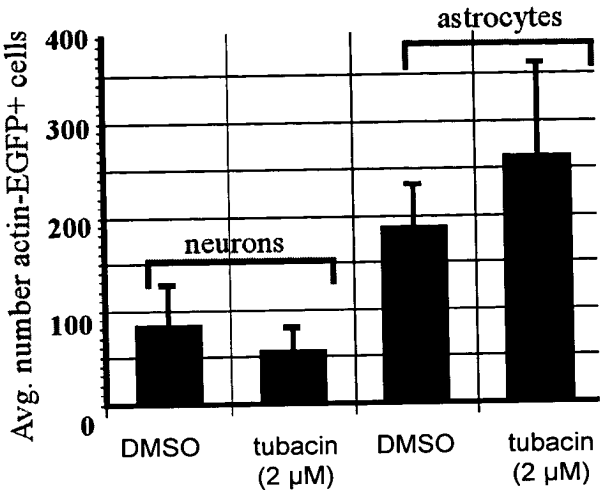


FIG. 50D